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Albert Szent-Györgyi Medical School
Doctoral School of Clinical Medicine

Innovative treatments in dermato-oncology: calcium electroporation and daylight photodynamic therapy

Ph.D. thesis

Dóra Ágoston M.D.

Supervisors: ERIKA KIS M.D., Ph.D. and ESZTER BALTAS M.D., Ph.D.

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LIST OF ABBREVIATIONS

AE = adverse event

AK = actinic keratosis

ALA = 5-aminolevulinic acid

ALM = acral lentiginous melanoma

ATP = adenosine-5-triphosphate

BCC = basal cell carcinoma

BD = block dissection

BRAF WT = BRAF wild type

Ca2+ = calcium ion

Ca-EP = calcium electroporation

CK AE1/AE3 = cytokeratin AE1/AE3

c-PDT = conventional photodynamic therapy

CR = complete remission

CTCA = Common Toxicity Criteria for Adverse Events

DNA = deoxyribonucleic acid

d-PDT = daylight photodynamic therapy

DSWC = dorsal skinfold window chamber

ECOG = Eastern Comparative Oncology Group

ECT = electrochemotherapy

EDF = European Dermatology Forum

EP = electroporation

ESOPE = European Standard Operating Procedures on Electrochemotherapy

5-FU = 5-fluorouracil

HPV = human papillomavirus

IFN = interferon

IQR = interquartile range

LED = light-emitting diodes

MAL = methyl 5-aminolaevulinate

MCC = merkel cell carcinoma

MM = malignant melanoma

NM = nodular melanoma

NMSC = non-melanoma skin cancer

NRS = numeric rating scale

OR = objective response

PD = progressive disease

PDT = photodynamic therapy

pT = primary tumour

PTP = permeability transitional pores

PpIX = protoporphyrin IX

PR = partial remission

PTP = permeability transition pores

QOL = quality of life

RECIST 1.1 = Response Evaluation Criteria for Solid Tumours 1.1

ROS = reactive oxygen species

RT = radiotherapy

SAE = serious adverse event

SCC = squamous cell carcinoma

SD = stable disease

SPF = sun protection factor

SSM = superficial spreading melanoma

UV = ultraviolet radiation

VAS = visual analogue scale

WHO = World Health Organization

1. INTRODUCTION

The treatment of dermato-oncology patients has undergone significant changes over the last decade [1]. A range of new agents were added to the therapeutic palette, targeting the whole spectrum of skin related malignancies, from precancerous conditions to metastatic form of skin cancers [1]. New therapeutic interventions have also emerged, and some methods have undergone incremental innovation. The changes affect almost all skin tumour types, from the most common keratinocyte-derived skin tumours to malignant melanoma (MM), and also include rare skin malignancies, like merkel cell carcinoma (MCC).

As a general principle, surgical excision under local anaesthesia remains the gold standard treatment of skin tumours diagnosed early. For locally advanced and metastatic forms, the determination of therapy is the task of a multidisciplinary team. In addition to surgical and radiotherapy, there is a wide range of new systemic treatments available, including molecular targeted and immuno-oncological therapies. In many cases, a favourable therapeutic response can be achieved by combining treatment modalities. More recently, the new systemic therapies have been increasingly used in the tumour-free setting as adjuvant treatment, to prevent or delay disease recurrence.

Keratinocyte-derived non-melanoma skin cancers (NMSC) include basal cell carcinoma (BCC) and cutaneous squamous cell carcinoma (SCC) which are the most common human tumours. The incidence has been increasing worldwide [2]. Early forms are treated by surgical excision, while radiotherapy and systemic treatments are preferred for locally advanced or metastatic tumours, and for patients presenting with multiple primary cancers. An alternative to standard treatments is electrochemotherapy (ECT), which uses electrodes to place the tumour in an electric field and delivers a cytostatic agent across the cell membrane to the tumour cells to be treated.

In the superficial and precancerous forms of keratinocyte tumours, surgery is not suitable in most of the cases. Therefore, different methods were developped to destroy these usually multiplex tumours, localised in the head and neck region [3].

Actinic keratoses (AK) are preinvasive cancerous lesions on sun-exposed skin, which may progress into invasive SCC. Currently there are no guidelines how to predict which AK will evolve into SCC, therefore their treatment is common practice. Local treatment alternatives for AK include cryotherapy, CO_2 laser, certain topical drugs (e.g. Imiquimod cream, 5-Fluorouracil cream) and photodynamic therapy (PDT). PDT is a non invasive medical

treatment utilising a topical photosensitizer, activated by light to exert cytotoxic activity in tumour cells.

For malignant melanoma the principles of treatment strategy are similar to those for NMSCs. Surgical excision is the first-line treatment for low-risk primary tumours and sentinel lymph node biopsy should be emphasised in the detection of occult lymph node metastases [1]. Block dissection can be offered to patients with occult lymph node metastases, and for clinically and/or radiologically detectable metastases. In the case of irresectable tumors and/or distant spread, systemic treatment is recommended in the form of molecular targeted or immuno-oncological therapy, which can be combined with other modalities, like radiotherapy or ECT.

Merkel cell carcinoma is a rare neuroendocrine skin tumour with aggressive biological behaviour [4]. The disease is prone to rapid progression and has an unfavourable prognosis. Surgical resection of the primary tumour and sentinel lymph node biopsy are the primary treatment, and adjuvant radiotherapy is of high priority. Among the systemic treatments, immunotherapy is recommended as first-line treatment [5-7]. ECT is a good option for local tumour control in MCC, which can be combined with immunotherapy, thereby increasing the efficacy of both therapies [8].

The use of electrochemotherapy and photodynamic therapy in the treatment of skin tumours started decades ago and has undergone significant incremental innovation, which continues today. In Hungary, both methods were first introduced at the Department of Dermatology and Allergology in Szeged, and are part of routine dermato-oncological care. The scientific work underlying this thesis presents the results obtained with the innovative versions of the two methods, calcium electroporation and daylight photodynamic therapy.

1.1. Electrochemotherapy

ECT is a repeatable procedure for the local treatment of primary and metastatic solid tumours. ECT was first used in the 1980s and the first successful clinical cases were described in 1991. Thanks to the European Standard Operating Procedures (ESOP) guidelines developed in 2006 and updated in 2018, it is now used in more than 200 oncology centres across Europe [9-19]. ECT was first introduced in our department in 2007.

1.1.1. Electroporation

ECT is based on the physical phenomenon of electroporation, which uses the increased permeability of the cell membrane due to an electric field to deliver molecules into the cell [12]. Short and strong electrical pulses increase the permeability of the cell membrane, allowing the introduction of different molecules into the cells, such as cytostatic drugs, which otherwise would not or only to a limited extent pass through the cell membrane.

During ECT, reversible electroporation is used. It means that during EP, high-intensity electrical pulses delivered by an electrical pulse generator cause destabilisation of the cell membrane that is only temporary, and stabilises within a few seconds [20]. Thus, large (hydrophilic) molecules can enter the cytosol through pores formed by transient fading of the phospholipid bilayer that forms the cell membrane, and become trapped in the cell by reorganisation of the cell membrane.

1.1.2. Chemotherapeutic agents used during electrochemotherapy

The most commonly used chemotherapeutic agents in ECT treatments are bleomycin and cisplatin. These agents' concentrations can be increased in the target cells by electroporation (8000-fold for bleomycin and 80-fold for cisplatin), thus increasing their cytotoxicity in the treated tumour [21, 22]. As a result, antitumour effect can be achieved with low-dose of chemotherapeutic agents, therefore side effects are minimised. In case of using bleomycin, the cell death is caused by the breakage of double-stranded desoxyribonucleic acid (DNA), while at cisplatin it is caused by the cross-linking of DNA.

1.1.3. Selectivity, vascular effect, immune response

With bleomycin, the fragmented chromosomes cause the cell to be unable to divide and then to die, meaning that the mitotically active cells, i.e. tumour cells and endothelial cells in the blood vessels supplying the tumour, are selectively destroyed, while the surrounding tissue is not. This cell-level selectivity explains the tissue-sparing property of ECT treatment [23, 24]. By destroying the endothelial cells of the blood vessels in the tumour, the procedure also has an antihemorrhagic effect. The antiangiogenic effect is predominantly exerted at the capillary level [25]. Since ECT involves non-thermal tumour ablation, tumour-specific antigens remain intact. The antigens released from the damaged, necrotic tumour cells activate T-cells, inducing an immune response and an abscopal effect on the tumour [26].

1.1.4. Advantages and disadvantages of electrochemotherapy

Comparing the benefits of ECT with other local ablative techniques, it is an effective, safe, tissue-sparing, cost-effective, low-risk and repeatable local treatment of solid tumours [11]. It can be used as palliative, curative, neoadjuvant or adjuvant intervention. Multiple tumours can be treated during one session of ECT, it is effective even after radiotherapy, and has been reported to improve patients' quality of life (QOL) [27]. Muscle contractions during EP can be uncomfortable, but adverse events are local and transient, including redness, oedema, ulceration, hyperpigmentation and moderate pain at the treatment site.

1.1.5. The use of electrochemotherapy in oncology

Its efficacy has been demonstrated in a number of tumours with different histological types, from superficial (basal cell carcinoma, squamous cell carcinoma, melanoma cutaneous metastasis, vulva carcinoma, parotid and thyroid tumour, MCC, cutaneous lymphomas) to deep-seated tumours (colorectal, prostate, pancreatic carcinoma and their metastases to the internal organs, brain tumours, bone metastases) and certain benign lesions (capillary malformation, keloid) [27]. In the largest prospective study published to date, an objective response (OR) of 85% and a complete response (CR) of 70% were achieved for superficially located tumours [28]. The efficacy of ECT in different histological types was as follows: malignant melanoma cutaneous metastases: OR 82%, CR 64%, basal cell carcinoma: OR 96%, CR 85%, breast cancer cutaneous metastases: OR 77%, CR 62%, squamous cell carcinoma: OR 80%, CR 63%, Kaposi's sarcoma: OR 98% CR 91%. In more than 400 ECT treatments performed in our department, regardless of the histological type of the tumour, an OR of 79.4% was observed, with CR in 49.4% of the treated tumours [27].

1.2. Calcium electroporation

1.2.1. The importance of calcium and its homeostasis

In addition to the chemotherapeutic agents mentioned above, many other drugs, radioactive compounds and calcium have also been tested in combination with EP. Calcium is an ubiquitous intracellular secondary messenger involved in many cellular processes such as gene transcription, proliferation, cell metabolism and apoptosis [29-35]. In eukaryotic cells, the intracellular calcium concentrations are lower than (10^{-7} mol/L) the extracellular concentrations (10^{-3} mol/L). Low intracellular calcium concentrations are required for calcium to function as a signal transduction molecule [36]. In contrast, calcium in high intracellular concentration can be cytotoxic. Preclinical studies have demonstrated that Ca-

EP can induce cell death *in vitro* and tumour necrosis *in vivo*, making Ca-EP a promising new antitumour treatment. In Ca-EP, calcium replaces bleomycin, the most commonly used chemotherapeutic agent in ECT, and may therefore be beneficial in patient populations in whom chemotherapeutic agents are contraindicated (e.g. patients with severe respiratory failure, pregnant women, etc.).

1.2.2. Mechanism of action of calcium electroporation

Ca-EP has similar properties to ECT. Ca-EP is a local treatment in which the intratumoral administration of calcium is followed by electrical pulses on the tumour. The electrical impulses delivered during EP help the calcium molecules to pass through the cell membrane into the cytosol. The influx of calcium into the cell is enhanced by increasing the extracellular calcium concentration before EP [37]. During Ca-EP, EP increases the intracellular calcium concentration, resulting in increased adenosine-5-triphosphate (ATP) consumption. In addition, an increase in mitochondrial membrane permeability results in a further decrease in ATP production. This ATP depletion, together with other effects on the cell, causes cell death (Figure 1.) [33, 38]. High calcium ion (Ca^{2+}) influx can lead to a decrease in ATP levels and cell death. First, increased intracellular Ca^{2+} levels increase the activity of Ca^{2+}-ATPase and Na^{+}/K^{+}-ATPase, which regulate Ca^{2+} homeostasis. Second, high Ca^{2+} concentrations enhance the opening of mitochondrial membrane permeability transitional pores (PTP) to dissipate the proton gradient. Impairment of the mitochondrial membrane electrochemical gradient results in mitochondrial collapse. When the mitochondria collapses, the cell can no longer produce ATP. Overall, increased ATP consumption and decreased ATP production leads to low ATP levels, which in combination with other cellular processes (lipase and protease activation, release of reactive oxygen species), results in cell death.

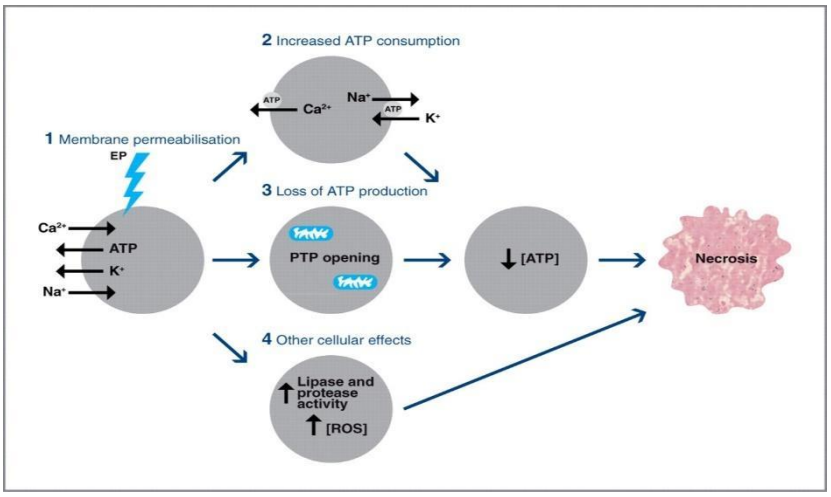

Figure 1. Calcium electroporation induces tumour necrosis [38].

The different sensitivity of normal and tumour cells to Ca-EP can be explained in part by membrane stabilisation after EP, by differences in the expression of calcium transporters and by changes in cellular structure. Calcium channels and proton pumps are present in malignant cells as in normal cells, but their function, localisation and/or activity may differ. EP is reversible, with the blockage of pores in the membrane reducing the flow of ions and molecules. The blockage of pores depends on temperature, the degree of permeabilization, the integrity of the cytoskeleton and the cell type. Normal cells appear to regenerate more rapidly compared to tumour cells, as confirmed by in vitro studies [39].

The vasoconstriction induced by EP causes local hypoperfusion, so during Ca-EP the injected calcium remains in the necrotized area and selectively kills tumour cells, as slower regeneration of the tumour cell membrane allows additional calcium influx into the cell. This explains why Ca-EP destroys tumour cells while leaving surrounding cells and tissues intact, as supported by preclinical studies [40].

Although Ca-EP is a local treatment, *in vivo* studies have demonstrated its enhanced systemic immune response inducing effect [26]. It induces immunogenic cell death by antigen release from the cytosol and antigen presentation across the cell membrane, alerting the immune system.

Its anti-angiogenic effects are confirmed by *in vitro* and *in vivo* studies. The *in vivo* vascular effects of Ca-EP in normal and tumour vessels were evaluated by intravitreal microscopy using a dorsal skinfold window chamber (DSWC) model [41]. The study demonstrated the vascular effects of Ca-EP on both normal and tumour vessels. *In vitro*, Ca-EP inhibited the formation of capillary-like structures and, similar to bleomycin-based ECT, damaged tumours as well as normal blood vessels. Larger blood vessels, arterioles and venules were

less affected and their functionality was preserved [40, 42]. Since the properties of Ca-EP are very similar to ECT, Ca-EP may be able to replace bleomycin with calcium in electroporation treatments.

1.3. Conventional and daylight photodynamic therapy

Photodynamic therapy is a non invasive medical treatment, which is based on the activation of light-sensitive molecules (photosensitizers) in the diseased tissues, resulting in the formation of reactive oxygen species, and leading to injury and cell deaths.

The most often used photosensitizers are 5-aminolevulinic acid (ALA) and its ester derivative methyl 5-aminolevulinate (MAL). Depending on the light source, PDT can be performed using artificial light or natural sunlight. The former is called conventional photodynamic therapy (c-PDT), while the latter daylight photodynamic therapy (d-PDT). Use of c-PDT in skin tumours was first reported in 1990 by Kennedy *et al.*, while d-PDT was first utilised in 2008 in Copenhagen by Wiegell *et al.* in the treatment of AK [43, 44]. We introduced c-PDT at the Department of Dermatology and Allergology in Szeged in 2003, while d-PDT is used since 2014 [45].

1.3.1. Mechanism of action of conventional and daylight photodynamic therapy

During PDT in dermatology, photosensitizers in a topical formulation are applied on the treated skin. These prodrugs will endogenously be converted by the heme biosynthetic pathway to protoporphyrin IX (PpIX) and other intermediate photosensitizing porphyrins [45]. The metabolite of the PpIX is excited by light of the appropriate wavelength, and all PpIX absorption peaks are within the visual spectrum of light. A range of light sources, including daylight can be used to activate the accumulated PplX like lasers, metal halide lamps, fluorescent lamps and light-emitting diodes (LEDs) [45]. During the return of PpIX to its ground state, reactive oxygen species are generated, resulting in cell death (necrosis or apoptosis) [46]. The photosensitizer accumulates faster and to a greater extent in rapidly dividing cells and newly formed blood vessels, than in the surrounding intact tissues, resulting in selective destruction of the tumour cells [47]. During c-PDT the illumination with light is performed after a predetermined three hours incubation period of the photosensitizer, when PpIX accumulation reaches its maximum. During d-PDT an incubation time for the photosensitizer cream of 30 minutes without occlusion is recommended, leading to a gradual accumulation of PpIX.

1.3.2. Advantages of daylight photodynamic therapy

The history of photosensitizer treatment dates back to antiquity and its continuous development still evolves today [46]. The modern era of PDT began in 1978, when Dougherty *et al.* performed the first systematic human trials of PDT in cutaneous and subcutaneous skin tumours [46]. PDT has undergone many innovations over the past decades [46]. The first photosensitizers were administered intravenously, and their effects lasted up to two months. Later more effective and less toxic ALA-based photosensitizers were developed for topical application, which are used today. Further research has focused on improving the stability of the formulations, easier and deeper penetration into the upper layer of the skin and more convenient application (transdermal patch) [46].

Nowadays, several topical preparations containing ALA or its methyl ester, methyl 5-aminolaevulinate (MAL) are indicated in dermato-oncology. The most important difference between the two agents is that PDT with the latter (MAL) is less painful. In those countries, including Hungary, where there is no topical photosensitizer registered on the market, an extemporary (magistral) cream formulation is prepared with the active substance.

Improvements of the intervention have also been made in the light used for PDT [46]. In addition to conventional PDT, d-PDT with natural sunlight has been introduced. C-PDT mostly uses an artificial light source emitting red (635 nm) or blue (410 nm) light to excite the photosensitizer. In our department, a light source emitting 630 nm red light (Aktilite®, PhotoCure ASA, Oslo, Norway) is used during c-PDT. D-PDT with natural sunlight (visible light: 380-700 nm) was first used in Copenhagen in 2008 by Wiegell and colleagues [44].

Literature data showed that the efficacy of d-PDT and c-PDT in the treatment of both superficial BCC and AK is similar, but d-PDT is better tolerated and associated with significantly less pain [46, 48]. Pain during illumination is the most common adverse event with c-PDT. Many patients with lesions on the head and neck find pain during illumination a remarkable problem. C-PDT involves incubation of the photosensitizer (ALA or MAL) in occlusion for several hours (3 h) in the treated area, leading to a high and selective accumulation of PplX in the target lesion. Irradiation with an artificial light source is performed when PpIX accumulation reaches its maximum. The international protocol for d-PDT recommends an incubation time for the photosensitizer of 30 minutes without occlusion. The accumulation of PpIX does not reach its maximum at the beginning of the exposure to sunlight, but continues gradually. This incremental PpIX activation is one possible explanation for the less pain during d-PDT compared to c-PDT [49]. Another advantage of d-PDT is, that it can be used to treat a large area of skin [46].

Additional factors limiting the use of c-PDT are the occlusion procedure and the lenghts of the treatment. The absence of occlusion and the use of daylight instead of an artificial light source result in a more simple procedure, where patients spend less time in the department. D-PDT is a simplified treatment with improved tolerability. It is as effective as c-PDT, but it is less painful, suitable for simultaneous treatment of a larger area of the skin, and time reducing for the health care [46, 48].

A disadvantage for d-PDT is, that it can be affected by certain weather conditions. Rainy and cold weather, strong winds hinder the treatment, while very sunny weather with high UV radiation is difficult to tolerate and increases the likelihood of unwanted effects (e.g. sunburn) [50]. An international consensus on d-PDT in AK was reached in 2012 and an European consensus in 2015 [51, 52]. Since then, several countries have introduced the method, adapting it to the geographical and climatic conditions of their region [52-56].

1.3.3. Place of photodynamic therapy in the treatment palette of actinic keratosis

PDT is a well recognized therapy in guidelines for AK, superficial BCC, and Bowen's disease [46, 51, 52]. The photosensitizers used during the intervention have these NMSCs in their label. However the procedure is also applied *off-label* for acne vulgaris, viral warts, condyloma, scleroderma, cutaneous leishmaniasis, cutaneous lymphomas and for skin rejuvenation.

Actinic keratosis is an *in situ* cutaneous squamous cell carcinoma with very high prevalence ranging from 40-60% in Australia, and in the Caucasian population over 40 years of age [2, 57]. Based on literature data, 60-80% of the invasive cutaneous SCCs arise from AK, therefore the treatment of AK is of high priority [58, 59]. Taking into account the strength of evidence, the treatment armamentarium for AK includes cryotherapy (high), 5-fluorouracil cream (5-FU) (high), imiquimod cream (high), PDT (moderate), CO_2 laser ablation (moderate) and diclofenac gel (moderate) [60, 61]. Surgical excision is recommended only in selected cases. Based on a systematic review published in 2021, the strongest recommendation for the treatment of AK was made for cryotherapy, 5-FU cream and imiquimod cream [3]. It has to be noted, that there is insufficient evidence on the efficacy of these treatments in immunosuppressed patients or their suitability for chemoprevention. Because on the phenomenon of field cancerization, there is often a need to treat more extensive areas of the skin (field-directed therapy). Based on the available data for field cancerization, the most suitable treatment options are PDT, local imiquimod cream and cryotherapy[3].

2. AIMS

2.1. Calcium electroporation in the treatment of cutaneous metastases (Table 1.).

Our objectives for the research	
Primary objective	Comparison of the efficacy of bleomycin-based ECT and Ca-EP in the treatment of cutaneous metastases of malignant tumours.
Secondary objective	Registering adverse events (AE/SAE) during Ca-EP and bleomycin-based ECT.

Table 1. Aims of the study: Ca-EP in cutaneous metastases (phase II, randomised, double-blind trial).

2.2. Daylight photodynamic therapy in the treatment of actinic keratoses (Table 2.)

Our objectives for the research	
Primary objective	Confirming the efficacy of d-PDT in the treatment of actinic keratoses under the local climatic conditions.
Secondary objective	Studying the safety and tolerability of d-PDT in the treatment of AK under the local climatic conditions, with particular focus on treatment-related pain.
Tertiary objective	Adapting a treatment protocol of d-PDT in the treatment of AK and introducing the method in our department.

Table 2. Aims of the study: daylight photodynamic therapy in AK.

3. PATIENTS AND METHODS

3.1. Calcium electroporation in the treatment of cutaneous metastases

3.1.1. Design of the clinical trial

The study protocol was approved and registered by the Centre for Health Registration and Education on 3rd of May 2016 under case number 032104/2016/OTIG. Our clinical trial was performed at the Department of Dermatology and Allergology, University of Szeged, with the permission of the Institutional and Regional Research Ethics Committee of Human Biomedical Sciences, University of Szeged (clinicaltrials.gov number: NCT03628417, registration date: 23 May 2016; permission number: 3806; registration number: 98/2016-SZTE; protocol number: ECT-KALCIUM-001).

3.1.2. Patients and methods

Inclusion and exclusion criteria

In our randomised, double-blind study we included patients with the following criteria (Table 3.).

Inclusion criteria	Exclusion criteria
Age > 18 years	Previous bleomycin treatment with more than 200,000 Units/m^2.
Verified cutaneous or subcutaneous metastases of any histology.	History of severe allergic reactions associated with bleomycin.
Platelet count ≥ 50 billion/L, INR < 1.5	Participating in other clinical trials involving experimental drugs or involved in a trial within 4 weeks prior to study drug administration.
ECOG performance status ≤ 2	Pregnancy and lactation
Expected survival > 3 months	Untreated coagulation disorder
At least one cutaneous metastasis between 0.5 to 3 cm and accessible to electroporation.	Treatment free interval of more than two weeks. However, patients treated with Navelbine, Capecitabine, or weekly paclitaxel can continue these treatments, if there is no regression of cutaneous metastases. Other medical cancer treatments such as endocrine treatment, targeted treatment and radiotherapy to another area may also continue.
Proper contraception	Limited capacity or incapacity
Signed informed consent	

Table 3. Ca-EP in cutaneous metastases: Inclusion and Exclusion criteria.

Treatment arms

A maximum of 10 cutaneous or subcutaneous metastases from 0.5-3 cm of any histological type were included per patient. One to 6 metastases (depending on the patient's number of metastases) were numbered 1-6 and randomised (ratio 1:1) into one of two treatment arms:

- Ca-EP
- Bleomycin-based ECT

The treatment of the remaining metastases (6-10) was known and these were used for biopsy sampling without evaluation of their clinical response.

Randomization

Randomization of metastases for each patient was performed using the nQuery Advisor 7.0 computer program. Randomization was performed by an independent clinical pharmacologist, who prepared and labelled syringes containing clear material according to the numbered metastases. The randomisation code was revealed 6 months after treatment.

Concentrations of the preparations used

- calcium chloride: 220 mmol/l (9 mg/ml) (based on preclinical data)
- bleomycin:1000 IU/ml (according to ESOPE guidelines)

Determination of tumour volume

ab^2 /6, where "a" = maximum diameter, "b" = maximum diameter perpendicular to "a"

Determination of the amount of drug injected into the tumour based on the tumour volume

Tumour < 0.5 cm^3 - 1 ml/cm^3 tumour volume

Tumour > 0.5 cm^3 - 0.5 ml/cm^3 tumour volume

Electrical pulses

The electrical pulses were delivered by a Cliniporator (IGEA, Carpy, Italy) according to the European Standard Operating Procedures on Electrochemotherapy (ESOPE) guidelines. Linear needle electrodes (8 pulses 400 V and 1000 V/cm, 0.1 ms duration, 5 kHz frequency) and hexagonal needle electrodes (4 pulses 730 V and 910 V/cm, 0.1 ms duration, 5 kHz frequency) were used according to the tumour location and size.

Type of anaesthesia

The single procedure was performed under local or general anaesthesia after a prior doctor-patient consultation.

3.1.3. Assessment of treatment outcomes and adverse events

Follow-up

The clinical study lasted 12 months. Tumour response at each follow-up visit (1-7, 15, 30, 60, 90, 180 and 360 days after treatment) was assessed according to the World Health Organization (WHO) Response Evaluation Criteria for Solid Tumours 1.1 (RECIST 1.1) criteria and photo-documentation was performed (Table 4.).

Clinical response assessment (RECIST 1.1.)
Complete remission (CR): complete regression of the treated tumour
Partial remission (PR): at least 30% reduction in the sum of lesion diameters compared to baseline
Progressive disease (PD): at least 20% increase in the sum of lesion diameters compared to baseline
Stable disease (SD): if none of the above categories are met
Objective response (OR): the sum of the total regression (CR) and the partial regression (PR)

Table 4. Assessment of clinical response according to RECIST 1.1.

Patients' Quality of Life (QOL) was assessed before and after treatment (0-100%). Pain was measured using the Numeric Rating Scale (NRS) (0-10 points: 0 = no pain, 1-3 = mild pain, 4-6 = moderate pain, 7-10 = severe pain). Potential adverse events were recorded using the Common Toxicity Criteria for Adverse Events version 4.0 (CTCA version 4.0).

Histopathological examination

Biopsies were performed from the tumour area before treatment and/or 7 days after treatment at patients with more than 6 (6-10) metastases, as described below (Table 5.):

Number of metastases included in the clinical trial (n)	**Number of clinically assessed metastases (n)**	**Number of biopsies before and/or 7 days after Ca-EP (n)**

1-6	1-6	0
7	6	1
8	6	2
9	6	3
10	6	4

Table 5. Number of metastases, clinically evaluated metastases and biopsies included in the clinical trial.

The randomization code was revealed six months after treatment and biopsies were taken from both calcium and bleomycin-treated lesions and analysed by our clinic's histopathologists for the presence of residual tumour tissue, inflammation, fibrosis and necrosis.

3.1.4. Statistical analysis

Statistical analyses were performed using IBM SPSS, v24, software and R statistical software. Objective tumour response (OR) was assessed using Fisher's exact test 6 months after treatment, Mann-Whitney test was used to measure the difference in the current delivered during Ca-EP and bleomycin-based ECT, and a two-sided 95% CI was used to compare the outcome of the two groups. The required number of tumours included in the clinical trial was determined by a non-inferiority test, with a minimum of 28 evaluable tumours at a significance level of 0.05 and a power of 80%. A non-inferiority cut-off of 20% was used to determine the clinical difference between the two treatment arms.

3.2. Daylight photodynamic therapy in the treatment of actinic keratoses

3.2.1. Design of the clinical trials

Our clinical trials with d-PDT were performed at the Department of Dermatology and Allergology, University of Szeged, with the approval and permission obtained from the Institutional and Regional Research Ethics Committee of Human Biomedical Sciences, University of Szeged (protocol number: PDT-DLIGHT; registration date: 4 Nov 2014; registration number: 137/2014).

A total of three clinical trials with d-PDT (PDT-DLIGHT-001, -002, -003) were conducted. Inclusion and exclusion criteria, assessment of treatment outcomes and adverse events were similar in all three trials. The treatment protocol in all studies was identical in the four major steps. Differences between studies were in the incubation time of the photosensitizer, and in the duration of time patient spent under natural sunlight. The treatment parameters suggested

by the international and European protocol were used as a basis, and changed step by step in order to optimize the method more tolerable for our patients under the local climatic conditions [46, 51, 52].

3.2.2. Patients and methods

In the studies with d-PDT we included patients who were older than 18 years. Other inclusion criteria were AK localised to the head and neck region, and the diameter larger than 6 mm. In our clinical trials, we primarily recruited patients presenting with a single AK. If there were multiple AKs, we assigned a target lesion, which was followed in the clinical trials for response and adverse events. The diagnosis of AK was based on the clinical picture and on the dermoscopic presentation. Histopathological verification of the skin lesions was performed only in selected cases.

We excluded from the studies those patients, who underwent medical intervention (cryotherapy, topical medication, surgical or laser treatment, radiotherapy) in the area of the AK six weeks prior to the study, and who had known hypersensitivity to the photosensitizing agent. After receiving detailed information on the procedure and purpose of the trial, patients confirmed their willingness to participate in the study by signing a consent form.

Photodynamic therapy with natural sunlight was performed in four steps. The skin area to be treated was prepared according to the international protocol [46, 51, 52]. Hyperkeratosis was removed from the AK with a Volkmann cannula. A high factor sunscreen was applied to the AK, and to the surrounding skin. Ten percent ALA was used as a photosensitizer in a magistral cream, and applied without occlusion to the AK and its 5 mm surrounding area. Light intensity was measured in the PDT-DLIGH-002 and 003 trials with the Vector H410 dosimeter (Scientech Inc. Boulder, CO, USA). The following formula was used to calculate the treatment dose or the time patients spent under daylight:

dose (J/cm^2) = [light intensity (mW/cm^2) × treatment time (min) × 0.6]/10.

The d-PDT treatment was postponed in case of rainy wheater conditions.

3.2.3. Assessment of treatment outcomes and adverse events

Clinical response was assessed based on the clinical and dermoscopic appearance six weeks after treatment at the follow-up visit. Complete remission (CR) was considered, if the tumour completely regressed, while a partial remission (PR) if at least one third of the lesion regressed. Progressive disease (PD) was defined as an increase in the size of the AK, while stable disease (SD) was defined when regression was seen in less than one-third of the lesion.

We did not investigate individual factors affecting the clinical response, and did not analyse statistically the association between clinical responses and treatment doses.

Adverse events were recorded using the CTCA version 4.0. Severity of AEs was rated according to a 5 point scale (0-4 points: 0 = none, 1 = mild, 2 = moderate, 3 = severe, 4 = very severe). Pain during treatment was measured on a visual analogue scale (VAS, scores 0-10: 0 = no pain, 10 = unbearable pain).

3.2.4. Statistical analysis

Statistical analysis in the d-PDT trials, as well as the comparison with the previous trial with c-PDT, were performed using IBM SPSS 26 software (IBM Corporation, Armonk, NY, USA). Data were not found to be normally distributed by one-sample Shapiro-Wilk test. Chi-square test was used to analyse the efficacy of PDT, and Kruskal-Wallis test to compare pain scores. Pairwise comparisons were performed using Dunn's post-hoc test. Comparisons of complete remission rates after the significant chi-squared test were performed using Benjamini-Hochberg correction. A threshold level of five percent was considered to indicate statistical significance.

4. RESULTS

4.1. Calcium electroporation in the treatment of cutaneous metastases

4.1.1. Evaluation of clinical response

A double-blinded randomised controlled trial was conducted in our institute to compare the efficacy of Ca-EP with bleomycin-based ECT in skin metastases (ClinicalTrials.gov: NCT03628417).

Seven patients (5 women, 2 men) with a total of 44 cutaneous metastases (34 melanoma malignum, 10 breast cancer) were included in the clinical trial between October 2016 and June 2018 (Figure 2.).

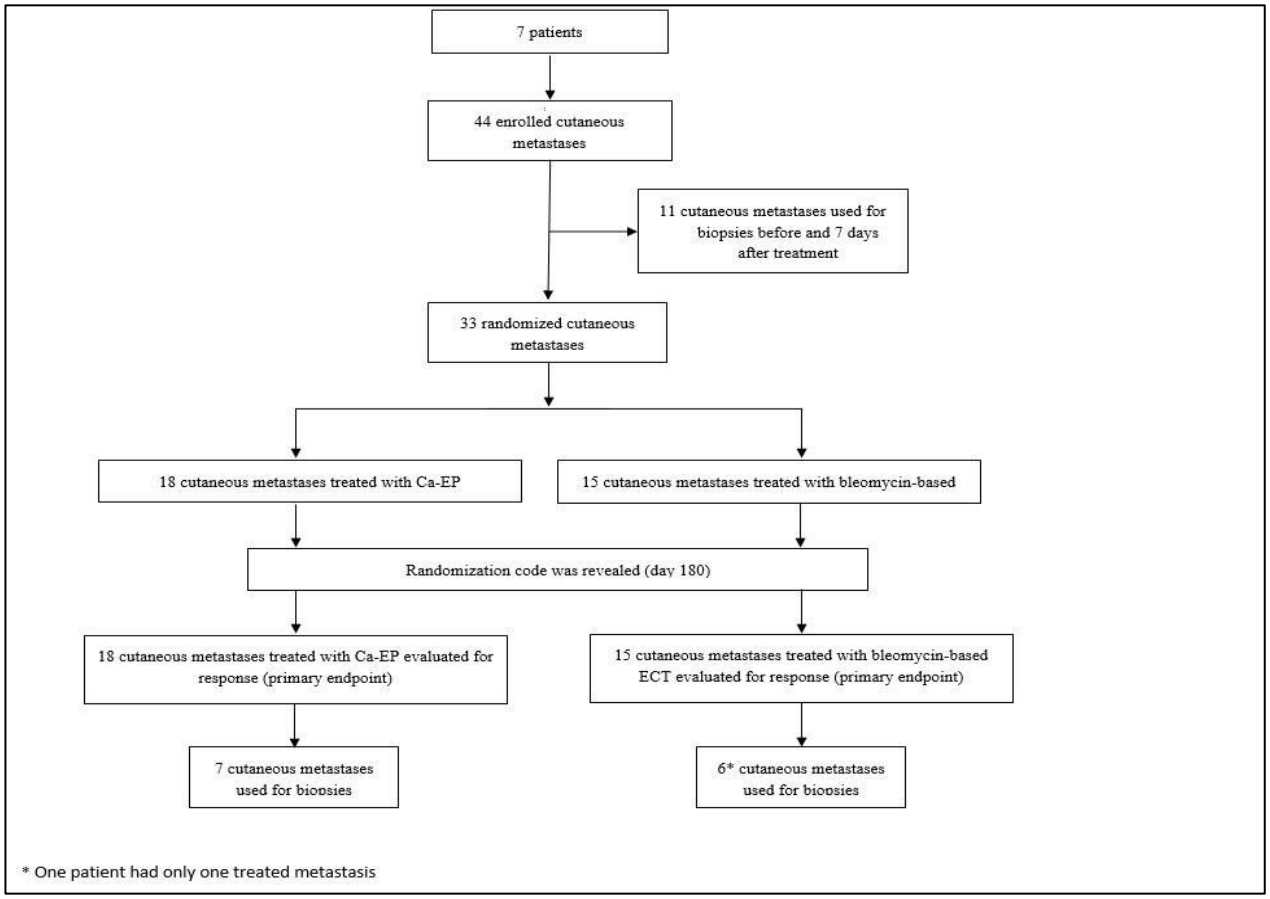

Figure 2. Illustration of trial profile.

Six patients with melanoma were treated for cutaneous metastases located on the lower extremity, while one patient was treated for breast cancer metastases localised to the trunk (Table 6.). The median age of the patients was 73 years (interquartile range: IQR = 21). 33 of the 44 metastases were randomised to the two treatment arms and their clinical response was evaluated, while 11 lesions were histologically analysed (Figure 2.). Of the randomised metastases, 18 were treated with Ca-EP (15 melanoma malignum, 3 breast cancer) and 15 with bleomycin-based ECT (12 melanoma malignum, 3 breast cancer). Of the 33 randomised cutaneous metastases, 6 (18%) were located in previously irradiated areas (2 lesions treated with Ca-EP, 4 lesions treated with bleomycin-based ECT). The median value of the maximum diameter of the 33 metastases evaluated by clinical response was 7 mm (IQR = 5). The median injected dose was 0.0855 mL (IQR = 0.1924) for Ca-EP and 0.132 mL (IQR = 0.27) for bleomycin-based ECT (Table 7.).

Patient	Gender Age (y)	Primary tumour (pT)	Localization of cutan mets.	Mets.included/ evaluated (n)	Years since diag.	Previous treatment
1.	Male (76)	MM (pT3b)	Lower extremity	1/1	1	-
2.	Female (62)	Breast cancer	trunk	10/6	5-6	systemic
3.	Female (83)	MM (pT3bN1)	Lower extremity	9/6	7	systemic, RT, ECT
4.	Female (49)	MM (pT3b)	Lower extremity	3/3	2	systemic
5.	Female (83)	MM (pT3a)	Lower extremity	10/6	4-5	ECT
6.	Female (64)	MM (pT2a)	Lower extremity	6/6	2-7.5	-
7.	Male (73)	MM (pT3a)	Lower extremity	5/5	3-8	systemic, RT
total	5F : 2M Mean age:70 (σ = 11-4891)	MM: 6 Breast cc:1	lower extr.: 6 trunk: 1	44/33	Mean: 3-8 (σ =1-9329)	various

Table 6. Baseline demographic and clinical characteristics of the patients.

Four of the procedures were performed under local anaesthesia and 3 under general anaesthesia.

Hexagonal needle electrodes were used for 21 (63.6%) and linear for 12 (36.4%) metastasis during EP. Of the 18 lesions treated with Ca-EP, hexagonal electrodes were used for 11 (61.1%) and linear electrodes for 7 (38.9%), while hexagonal electrodes were used for 10 (66.7%) and linear electrodes for 5 (33.3%) of the 15 lesions treated with bleomycin-based ECT (Table 7.).

Treatment arm	Calcium electroporation			Bleomycin-based electrochemotherapy		
	Our clinical trial	**2018 Study**	**Total**	**Our clinical trial**	**2018 Study**	**Total**
Lesion Characteristics						
Tumour size						
Median of the largest diameter, mm	6-5 (5-30)	9-5 (5-18)		7 (5-25)	11 (4-25)	
Tumour type						
Malignant melanoma	15	1	16	12	1	13
Breast cancer	3	17	20	3	18	21
Previously irradiated lesions, n	2	8	10	4	7	11
Location						
Lower extremity	15	4	19	12	4	16
Trunk	3	14	17	0	15	15
Upper extremity	0	0	0	3	0	3
Treatment						
Median doses (range), mL	0-085 (0,042-3,14)	0-24 (0,03-1,21)		0,132 (0,065-0,475)	0,21 (0,03-0,55)	
Median delivered current (range), A	3,85 (1,4-9)	3-4 (0,9-8,2)		4 (1,4-6,5)	2,8 (1-9,6)	
Median delivered current with linear electrodes (range), A	4 (2,25-9)	3-4 (0,9-8,3)		5,05 (4-6,1)	2,8 (1-9,6)	
Median delivered current with hexagonal electrodes (range), A	2-5 (1,4-4,2)	NA		2,75 (1,4-3,6)	NA	
Median number of applications (range), n	1 (1-6)	3 (1-7)		1 (1-3)	3 (1-7)	
Electrodes						
Linear	39% (7)	100% (18)		33% (5)	100% (19)	
Response (CR) for linear electrode subgroup	14% (1)	66% (12)		0	68% (13)	
Hexagonal	61% (11)	0		67% (10)	0	
Response (CR) for hexagonal electrodes subgroup	27% (3)	NA		60% (6)	NA	
Clinical response						
Complete response, percent, n	4	12	44-44% (16)	6	13	55-88% (19)
Partial response, percent, n	2	1	8-33% (3)	2	3	14-7% (5)
Stable disease, percent, n	6	3	25% (9)	5	0	14-7% (5)
Progressive disease, percent, n	6	2	22-22% (8)	2	3	14-7% (5)
Adverse events						
Ulceration, percent, n	2	7	25% (9)	3	13	47-05% (16)
Itch, percent, n	0	1	2-77% (1)	0	5	14-7% (5)
Hyperpigmentation, percent, n	2	0	5-55% (2)	6	5	32-35% (11)
Exuding, percent, n	0	2	5-55% (2)	0	2	5-88% (2)

Table 7. Results of our clinical trial and comparison with the first phase II randomised double-blind trial by Falk *et al.* in 2018 [62].

Clinical response assessment

The OR for Ca-EP was 33% (CR = 22%; PR = 11%) and for bleomycin-based ECT was 53% (CR = 40%; PR = 13%) (Table 7.). There was no significant difference in either OR (p = 0.30) or CR (p = 0.45) between the two treatment arms.

After six months, 33% (6 out of 18) of metastases had progressed in calcium-treated lesions compared to 13% (2/15) of bleomycin-treated lesions. The two-sided 95% CI for the difference in outcome between the two treatment arms was -13.3-53.3%. There was no significant difference in the clinical response of lesions located in previously irradiated areas compared to other cutaneous metastases (p = 0.37) (Figure 3.).

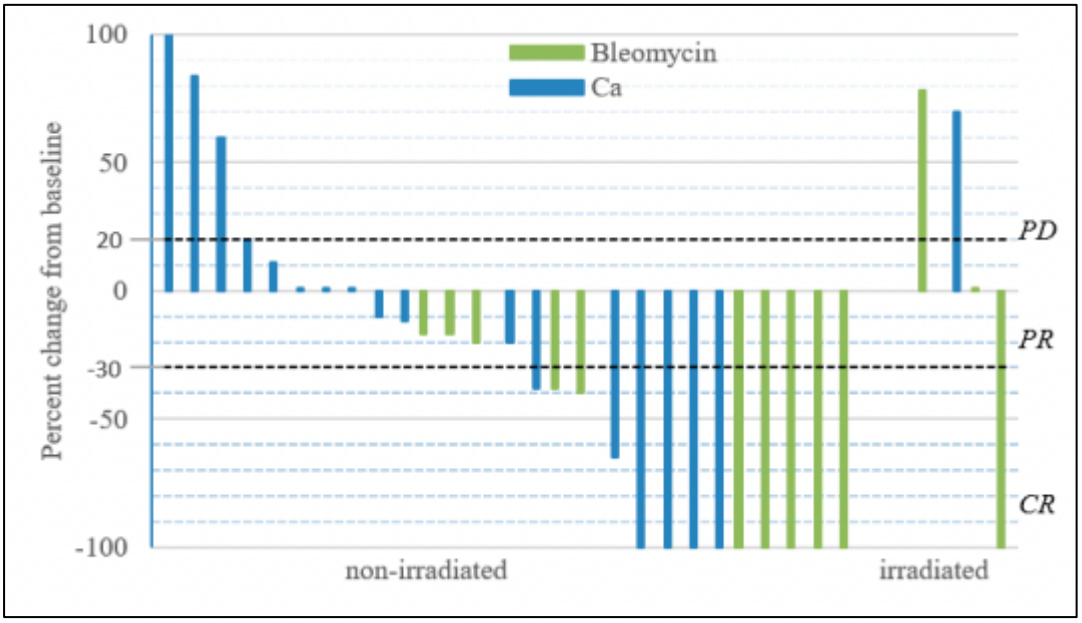

Figure 3. Changes in tumour size over time.
Metastases were treated at day = 0 with either i.t. calcium and or i.t. bleomycin in a randomised double-blinded study design. Patients received only one treatment and response was evaluated 6 months after treatment, after the randomization code was revealed. Change in size over time; the graph illustrates the percent change in tumour size recorded 6 months after treatment. The two non measurable metastases treated with calcium-chloride and bleomycin were irradiated, and are not included in the graph, but were included in the response analysis as PD.

Presentation of the histological results

Before and 7 days after treatment, 11 cutaneous biopsies were taken from the cutaneous metastases (Figure 4.).

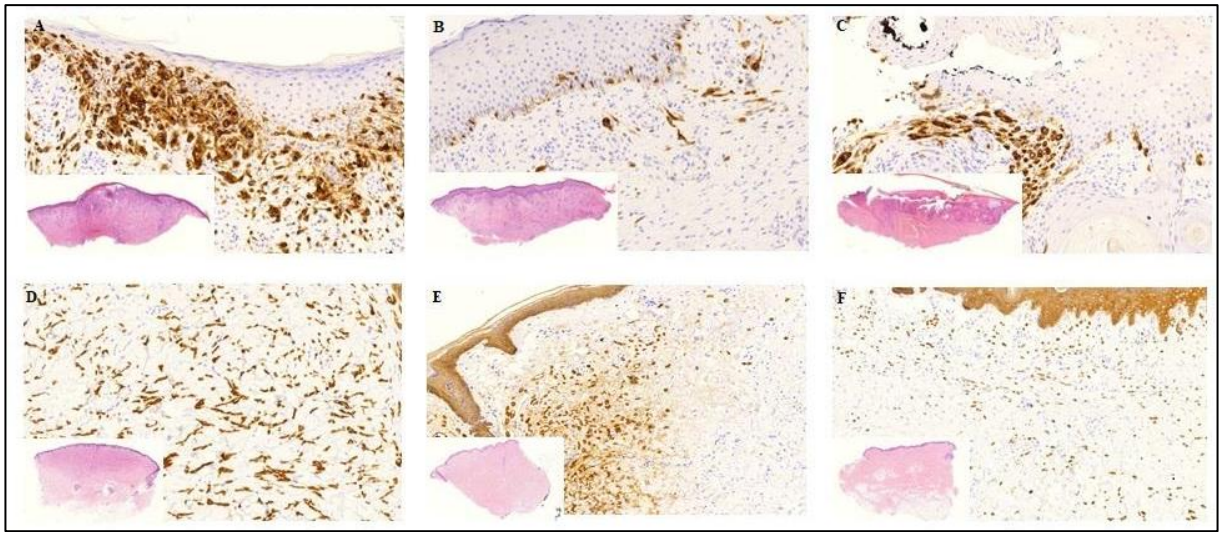

Figure 4. Histological description of biopsies taken before and 7 days after treatment.
Patient no. 5 with MM. (A–C)
(A): Pre-treatment biopsy: Extensive tumour infiltration, mild fibrosis, moderate lymphocytic inflammation, no necrosis. The tumour cells show diffuse MelanA positivity.
(B) Day 7. Post-treatment with Ca-EP: Partly ulcerated skin, moderate tumour infiltration and fibrosis, mild inflammation, no necrosis. Only scattered MelanA positive tumour cells.
(C) Day 7. Post-treatment with bleomycin-based ECT: Partly fragmented, ulcerated skin with pseudoepitheliomatous hyperplasia of the epidermis, moderate fibrosis and inflammation, no necrosis. Focal MM nests with MelanA positivity.
Patient no. 2 with breast cancer. (D–F)
(D) Pre-treatment biopsy: Extensive breast cancer infiltration without fibrosis, inflammation and necrosis. The tumour cells are cytokeratin (CK) AE1/AE3 positive.
(E) Day 7. Post-treatment with Ca-EP: Focal tumour infiltration, very mild inflammation no fibrosis or necrosis.
(F) Day 7. Post treatment with bleomycin-based ECT: Dispersed tumour cells with CK AE1/AE3 positivity, mild inflammation, no fibrosis or necrosis.

Out of the 13 histological samples taken 6 months after treatment, 6 (3 lesions treated with Ca-EP, 3 lesions treated with bleomycin-based ECT) showed clinical CR, based on the randomization code. In 5 of the 6 cases, no tumour cells were detected, so that CR could be confirmed by histopathological examination (3 lesions treated with Ca-EP and 2 lesions treated with bleomycin-based ECT) (Figure 5.).

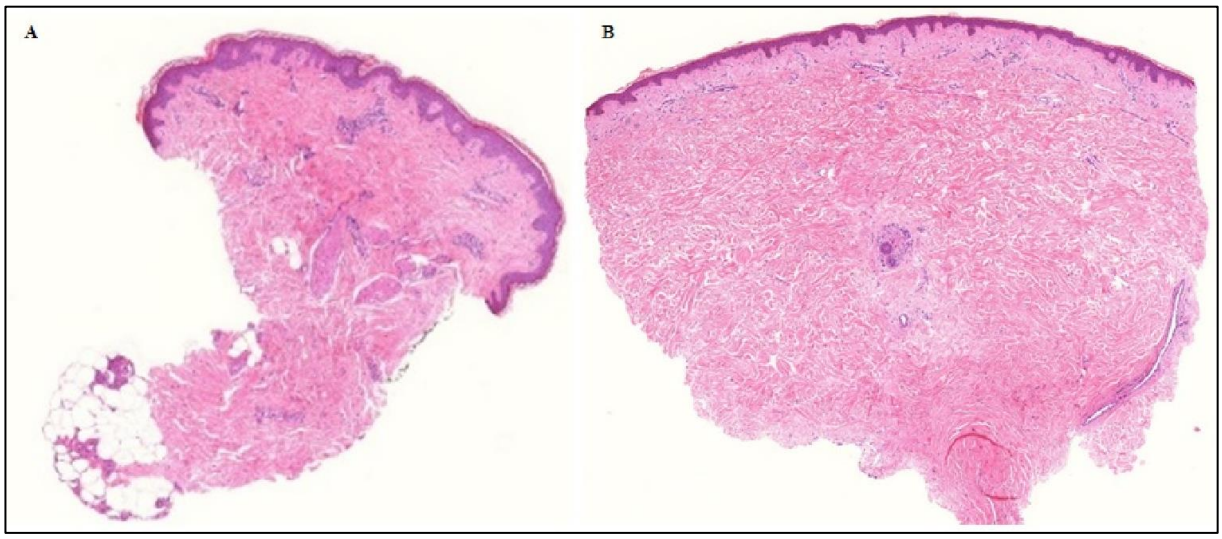

Figure 5. Histopathologically confirmed complete remission 6 months after Ca-EP.
Tumour cells were not identified 6 months after Ca-EP neither in malignant melanoma (A) nor in breast cancer metastases (B).
A: patient no. 3, Ca-EP treated clinically CR of melanoma malignum cutaneous metastasis.
B: patient no. 2, Ca-EP treated clinically CR of breast cancer cutaneous metastasis.

4.1.2. Summary of the physical parameters of the treatment

Electrodes used

Differences were observed in tumour response and electrode type between the two treatment arms, which were not statistically significant due to the small sample size. OR values were higher in Ca-EP with linear electrodes (42.8%, 3/7) than with hexagonal electrodes (27.3%, 3/11) ($p = 0.63$). 45.45% (5/11) of tumours classified as PD were treated with hexagonal electrodes and 14.29% (1/7) with linear electrodes ($p = 0.32$). The opposite trend was observed with bleomycin-based ECT. For lesions with OR, 70% (7/10) were treated with hexagonal electrodes and 20% (1/5) with linear electrodes ($p = 0.12$). 10% (1/10) of tumours classified as PD were treated with hexagonal electrodes and 20% (1/5) with linear electrodes ($p = 1$).

Applied current

There was no significant difference in the delivered currents between the two treatment arms ($p = 0.956$), nor between metastases treated in previously irradiated and non-irradiated areas ($p = 0.911$). The median delivered current was 3.85 A (IQR = 3.75) for metastases treated with calcium and 4 A (IQR = 2.4375) for metastases treated with bleomycin. The median current applied in the treatment of metastases in non-irradiated areas was 3.85 A (IQR =

3.45) and 3.95 A (IQR = 1.525) in previously irradiated areas. The electrical pulses were delivered a total of 30 times (median: 1; 1-6; IQR = 1) for the 18 randomized Ca-EP-treated metastases and 22 times (median: 1; 1-3; IQR = 1) for the 15 bleomycin-based ECT-treated lesions. The median value of current intensity measured with linear electrodes in the two treatment arms (Ca-EP and bleomycin-based ECT) was 5 A (2.25-6.1; IQR = 2.1; 31 applications) and 2.5 A (1.4-4.2; IQR = 1.6; 21 applications) with hexagonal electrodes. The median current delivered during Ca-EP was 2.5 A (1.4-4.2; IQR = 1.5; 11 applications) with hexagonal and 4 A (2.25-9; IQR = 3.5; 19 applications) with linear electrodes. With bleomycin-based ECT, the median current delivered in the group was 2.75 A (1.4-3.6; IQR = 1.7125; 10 applications) using hexagonal and 5.05 (4-6.1; IQR = 1.325; 12 applications) using linear electrodes (Table 7.).

4.1.3. Adverse events during treatment

No serious adverse events were observed. Based on the CTCA 4.0 criteria system, grade I adverse events such as ulceration and hyperpigmentation after Ca-EP were observed in 2-2 metastases (2/18, 11%). Following bleomycin-based ECT, ulceration occurred in 20% (3/15) and hyperpigmentation in 40% (6/15) of treated lesions (Figure 6.).

The median NRS score for pain before treatment was 2 (IQR = 2). The median NRS score immediately after treatment was 2 (IQR = 9), with 3 patients reporting no pain (NRS: 0), 1 reporting mild pain (NRS: 1-3) and 1 reporting moderate pain (NRS: 4-6).

Two patients with more than 6 cutaneous metastases who underwent biopsy reported severe pain (NRS: 9-10). The median 6-month NRS score was 2 (IQR = 4), with a mean Ca-EP of 2.5 (σ = 3.2016) and 4.5 (σ = 2.2913) for patients treated with ECT.

At six months after treatment, QOL scores were the same or increased from baseline (from 70% (IQR = 10) to 80% (IQR = 10). Three patients experienced no change in their quality of life at 6 months after treatment. All 7 patients agreed to repeat treatment in the future if necessary.

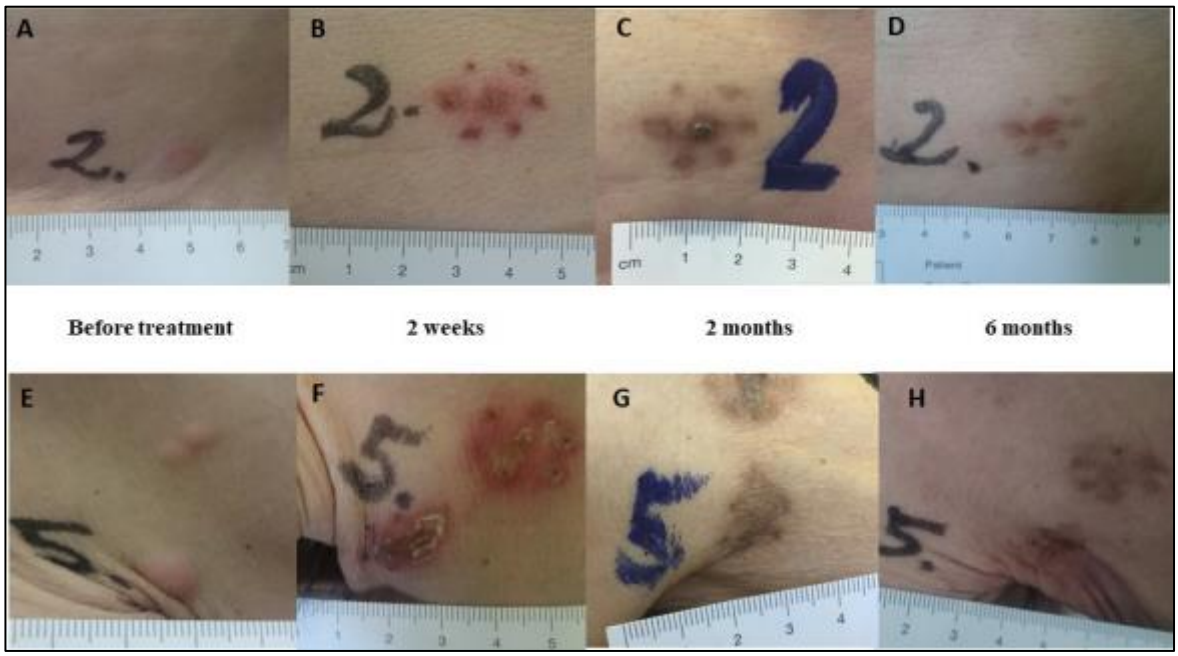

Figure 6. Evaluation of clinical response after Ca-EP and bleomycin-based ECT.
The lesions are from patient no. 2 with cutaneous metastases from breast cancer in the same region (trunk). Lesion no. 2: Ca-EP treated cutaneous metastasis. Lesion no. 5: bleomycin-based ECT treated cutaneous metastasis.
(A, E): Before treatment. (B, F): Two weeks after treatment; typical crust appearance. (C, G): Two months after treatment; clear hyperpigmentation in the areas treated with calcium and bleomycin. (D, H): Six months after treatment; complete disappearance of metastases.

4.1.4. Follow-up data

The mean follow-up of 6 patients lasted 29 months (standard deviation = 6.8232). One patient died 11 months after treatment (due to progression of another known primary tumour) and two patients died 26 and 27 months after treatment due to femoral neck fracture and malignant progression of melanoma, respectively. None of the lesions showing CR recurred during the 1-year follow-up period.

4.2. Daylight photodynamic therapy in the treatment of actinic keratoses

4.2.1. Evaluation of treatment outcomes and adverse events

A total of three clinical trials with d-PDT (PDT-DLIGHT-001, -002, -003) were conducted (Table 8.).

In the PDT-DLIGHT-001 study, the photosensitizer was incubated without occlusion on the skin for 30 minutes according to the international protocol. Afterwards, patients were exposed to sunlight in the open air for 90 minutes [63]. A total of 63 patients (33 male, 30 female) with AK (grade I) were treated in the head and neck region (face, forehead, nose) (Figure 7.). The mean age of the patients was 75.37 years (range 49-92 years). One hour

before treatment, the mean outdoor temperature was 25.63°C (12-32°C). Treatment was performed under cloudy (1-13 °C), partly sunny (10-25 °C) and sunny (17-31 °C) weather conditions. 73% of AKs (n = 46) showed complete remission (CR) and 27% (n = 17) showed partial remission (PR). Pain during treatment was rated by the patients as 0.3 (0-5) on the VAS **(**Table 8.).

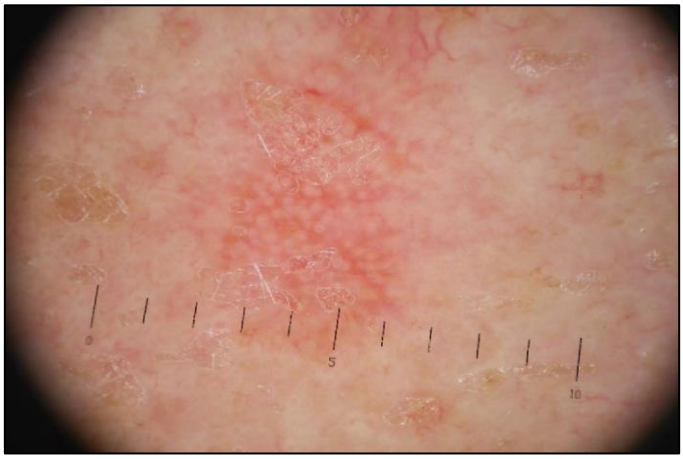

Figure 7. Dermoscopic image of AK (grade I) in one of our patient.
Large follicles are filled with yellow keratin plug and surrounded by a whitish halo, and a pink network of blood vessels is present („strawberry pattern").

In the PDT-DLIGHT-001 study, we observed that spending 90 minutes outdoors under sunny and partly sunny weather conditions was difficult to tolerate for our elderly patients. Taking this into account, in our next study (PDT-DLIGHT-002) we increased the duration of the incubation time of the photosensitizer, which patients spent indoors, while shortened the treatment time outdoors. After application of the photosensitizer cream, we waited 120 minutes instead of 30 minutes to allow PpIX accumulation. Patients stayed inside the clinic building during the incubation period (between 9 and 11 a.m.) and then were exposed to natural sunlight in the garden of our clinic for 30 minutes. The treatments took place in cloudy (18-29°C), partly sunny (19°C) and sunny (25-35°C) weather conditions.

The PDT-DLIGHT-002 study included 30 patients (16 men, 14 women) with an average age of 77.9 years (41-97 years). The average natural sunlight intensity measured with a dosimeter (Vector H410) prior to treatment was 48.63 mW/cm² (7-71 mW/cm²). Patients spent 30 minutes outdoors and the average treatment dose was 87.5 J/cm² (12.6-127.8 J/cm²) (Table 8.). When the treatment efficacy was evaluated after 6 weeks, 63.33% (n = 19) of AKs showed CR and 36.66% (n = 11) PR. Patients tolerated the therapy well, with a mean treatment pain score of 2.13 points on the VAS scale. The pain was mild, resolved

spontaneously, and no treatment interruption was necessary. Thirty-three percent of patients (n = 10) experienced severe erythema within 24 hours of treatment and presented in our department for treatment.

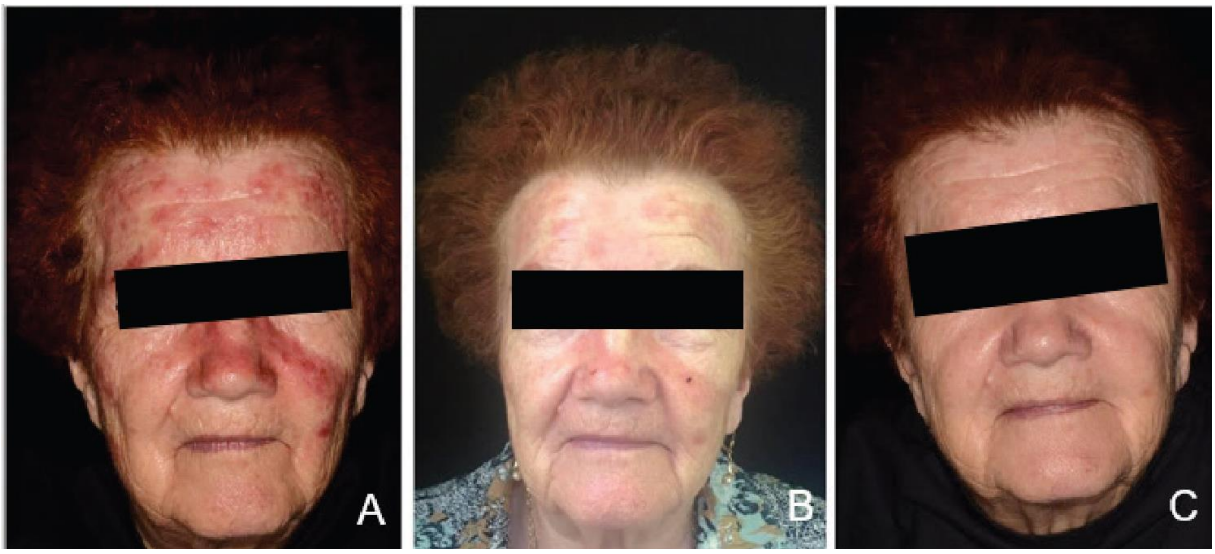

Figure 8. Severe erythema following high-dose d-PDT in a 79-year-old female patient.
(A) Severe erythema 24 hours after d-PDT of multiple AKs (dose: 108 J/cm^2).
(B) The erythema was reduced after 5 days of topical treatment.
(C) No erythema was observed at the 6-week follow-up, with complete remission of AKs.

With the application of topical skin soothing treatment, the erythema subsided within a few days and then disappeared completely (Figure 8.). When analysing the data, we found that these patients were treated under sunny weather conditions with higher doses (above 100 J/cm²). In our study, lesions treated with doses below and above 15 J/cm^2 showed no difference in clinical response (CR and PR).

The PDT-DLIGHT-003 clinical trial used dosimetry to measure light intensity. Considering the severe erythema seen at doses above 100 mJ/cm^2, we aimed to use lower treatment doses in. We included 73 patients (47 males, 26 females) with a mean age of 74.64 years (51-92 years). The mean light intensity was 46.67 mW/cm² (2-92 mW/cm²). The mean outdoor treatment time was 10.42 min (2-60 min). During treatment, patients received an average dose of 19.47 J/cm² (7.2-54 J/cm²) (Table 8.). There was no pain or severe erythema during the treatment. At the 6-week follow-up, 85% (n = 62) of the treated AKs showed CR and 15% (n = 11) PR.

Patient and treatment characteristics, results	PDT-DLIGHT			c-PDT [64]
	001	002	003	
Number of patients (n)	63	30	73	22
Sex (male to female) (n)	33:30	16:14	47:26	11:11
Average age (years)	75,37 (49-92)	77,90 (41-97)	74,64 (51-92)	75,86 (62-92)
Photosensitizing agent	10% ALA			20% ALA
Indoor incubation time (min)	30	120	120	240
Outdoor treatment time (min)	90	30	10,42 (2-60)	12 min/field
Measured brightness (mW/cm^2)	NA	48,63 (7-71)	46,67 (2-92)	NA
Treatment dose (J/cm^2)	NA	87,5 (12,6-127,8)	19,47 (7,2-54)	37
Complete Remission (CR) % (n)	73,01 (46)	63,33 (19)	84,93 (62)	59,09 (13)
Partial Remission (PR) % (n)	26,98 (17)	36,66 (11)	15,07 (11)	31,82 (7)
Stable disease (SD) % (n)	0	0	0	9,09 (2)
Progressive disease (PD) % (n)	0	0	0	0
Pain (VAS scale: 0-10)	0,3 (0-5)	2,13 (0-10)	0	6,94 (3-10)
Patients under pain relief (n)	0	0	0	21

Table 8. Patient characteristics, treatment parameters and outcomes in studies with d-PDT and c-PDT [64].

4.2.2. Comparing conventional and daylight photodynamic therapy

We compared the results of the recently performed studies with d-PDT and the earlier conducted investigations with c-PDT in our departement [64]. There was no significant difference in efficacy between treatments based on the international d-PDT protocol used in our PDT-DLIGHT-001 trial and the modified protocol used in PDT-DLIGHT-003 ($p = 1$). The d-PDT performed according to the PDT-DLIGHT-003 study protocol was significantly more effective compared to c-PDT in the prevoius trial by Gaál *et al.* ($p = 0.003$, khi-square test) (Figure 9.). The khi-square test showed a significant difference in CR rates between the four groups ($p = 0.003$), with the PDT-DLIGHT-003 protocol leading to the highest proportion (84.9%) of patients reaching CR. A significant chi-squared test for comparison of complete remission rates showed a significant difference ($p = 0.045$) between the PDT-DLIGHT-003 protocol (84.9%) and the c-PDT protocol (59.1%) with Benjamini-Hochberg correction, favouring d-PDT.

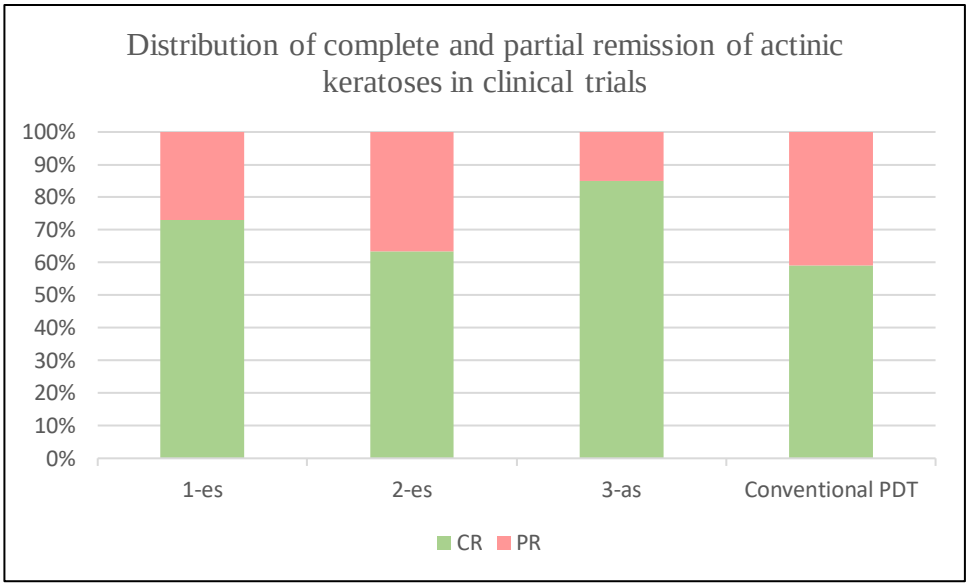

Figure 9. Complete and partial remission in AK with d-PDT and c-PDT.

Statistical analysis showed that there was a significant difference between the pain scores in the four groups ($p < 0.001$, Kruskal-Wallis test). Dunn's post-hoc analysis showed that the pain scores were significantly higher ($p < 0.001$) in the c-PDT previously performed in our department by Gaál M. *et al.*, than in our current d-PDT studies (Figure 10.).

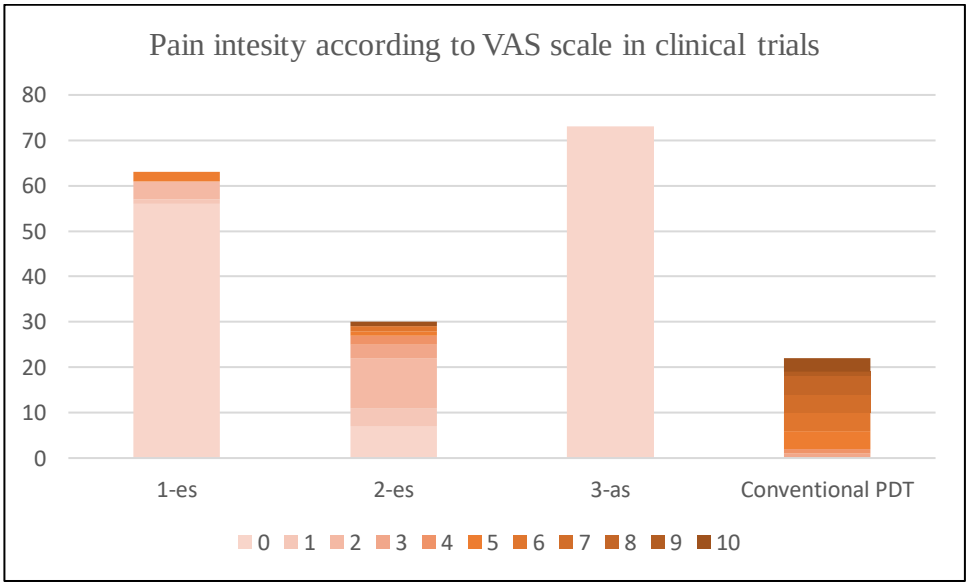

Figure 10. Pain scores on the VAS scale with d-PDT and c-PDT. Darkening of the colour indicates increasing pain.

4.2.3. Adaptation of the international treatment protocol of daylight photodynamic therapy

We conducted three clinical trials with d-PDT. In the PDT-DLIGHT-001 trial using the international treatment protocol for dPDT, we achieved complete remission in 73% of AKs and partial remission in nearly 27%. For our elderly patients the procedure was difficult to tolerate, because they had to spend 90 minutes under natural daylight. Our protocol was subsequently modified, and we changed the proportion of time spent indoors and outdoors, while the overall length of treatment did not change significantly (120 minutes versus 150 minutes).

In the PDT-DLIGHT-002 trial we achieved similar efficacy with the modified protocol, CR in two third and PR in one third of AKs. However, d-PDT with a 30 minutes outdoor treatment time resulted in adverse events. 30% of our patients presented to our department with severe erythema within 24 hours of treatment. Analysis of treatment doses showed that severe erythema occurred in patients who received doses above 100 J/cm². Our protocol was further modified in order to avoid high treatment doses. We incorporated dosimetry to measure the light intensity and calculated the treatment time patients needed to spend outdoore. In our subsequent study (PDT-DLIGHT-003), the average treatment dose was around 20 J/cm² and the average treatment time was around 10 minutes. We observed complete remission in 85% of AKs and partial remission in 15%.

Based on previous protocols and on our own findings, after step by step modification we have developed a local protocol for d-PDT in Szeged and a detailed explanation with pictures about the procedure for patients (Table 9., Figure 11.). We introduced the local protocol for d-PDT in AK into the daily dermatological practice at our departement.

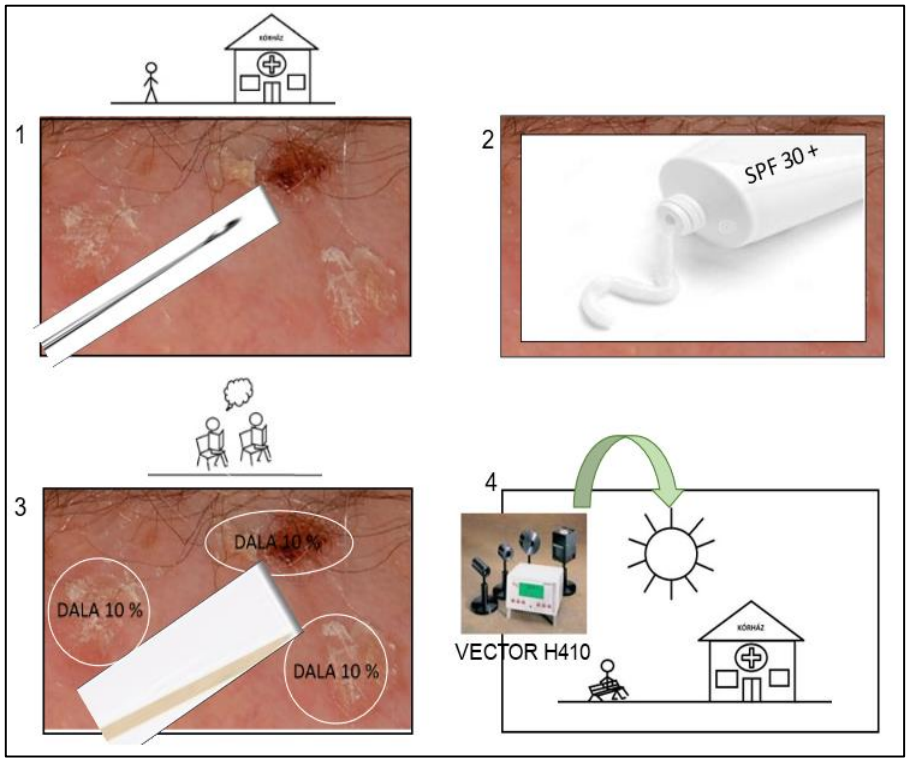

Figure 11. Explanation of the d-PDT treatment for patients with pictures.

Steps of the treatment		Details
1.	Preparation of the treatment area on the skin.	Remove the hyperkeratosis from the treatment area by curettage to facilitate the penetration of the photosensitizing agent into the skin.
2.	Application of a sunscreen.	Apply on the treated and surrounding area chemical sunscreen with high SPF (30+).
3.	Application of the photosensitizing drug.	Apply 10% ALA cream in a thin layer on the treatment area, without occlusion. Incubation time indoors is 120 min.
4.	Illumination by natural sunlight.	Use dosimetry to measure daylight intensitiy, and avoid high treatment doses (100 J/cm^2). (Keep average outdoor time in summer around 10 min.) Rain, temperature 10 °C↓ or 35 °C↑: d-PDT has to be postponed.
5.	Removal of photosensitizer and after-care.	Remove the photosensitizer form the skin surface following treatment. Advice skin soothing cream and sun protection.

Table 9. Simplified local treatment protocol in Szeged for d-PDT in AK.

5. DISCUSSION

5.1. Calcium electroporation in the treatment of cutaneous metastases

Ca-EP is a method for the treatment of local solid tumours in which short, high-voltage electrical pulses delivered to the tumour after intratumoral administration of calcium temporarily permeabilize the cell membrane, facilitating the passage of calcium molecules into the cell.

Effect of calcium electroporation on tumour cells (in vitro, in vivo)

Ca-EP has been tested in vitro on 18 different cell lines (12 tumour and 6 normal cell lines), all of which have shown a cell death inducing effect of calcium in combination with EP [34, 37, 38, 40, 65-68]. Ca-EP has similar effects to bleomycin-based ECT, but no synergistic effect is observed when calcium and bleomycin are combined before EP [33].

When calcium was applied at individual concentrations up to 5 mM, the treatment had no effect, and no change was observed at levels up to 20 mM in 2 cell lines. Although some effects were observed with bleomycin treatments, the magnitude of these effects was much smaller than when combined with electroporation [40, 42]. *In vivo* studies of Ca-EP were performed on subcutaneous tumours induced in immunodeficient mice in 5 different human tumour types (bladder cancer, breast cancer, colon cancer, small cell lung cancer and rhabdomyosarcoma). In addition, tumours (colon adenocarcinoma, melanoma) from immunocompetent or immunodeficient rodents have also been investigated [40, 67].

Cell death occurred in all the tumours tested, but with different sensitivity. Small cell lung cancer was found to be the most sensitive (CR = 89%) of the tumours treated in immunodeficient mice [23]. Colon tumours treated in immunocompetent mice were more sensitive (CR = 100%) compared to those treated in immunodeficient mice [67], suggesting a role for Ca-EP-induced immune response.

Ca-EP and its effects on normal skin and muscle tissues were tested *in vitro* on 6 normal cell lines. The difference between the effects of Ca-EP on normal and tumour muscle cells were compared in two in vitro studies. The rate of cell death was significantly lower in normal muscle cells compared to tumour cells [68, 65]. The extent of cell death showed significantly lower values in normal muscle cells compared to cancer cells. In *in vitro* studies in a suspension of normal human umbilical vein endothelial cells, Chinese hamster ovary cells and human primary dermal fibroblasts, the Ca-EP induced cell death in normal cells [37,

40]. In studies of human breast, bladder and colon cancer (3D spheroid), cell death occurred in all three cases with less effect on normal cells [36]. The results were confirmed in *in vivo* studies in mice, where normal cells around the tumour were less affected compared to tumour cells treated with Ca-EP [23]. Similar to what was observed in ECT, normal cells are less sensitive to Ca-EP than tumour cells [24]. The extracellular ATP store in both normal and tumour cells is depleted within 72 hours, which normal cells are able to survive. As with ECT, *in vitro* and *in vivo* studies have shown that Ca-EP exerts a vascular effect on both normal and tumour blood vessels [25, 40, 41]. The resulting vascular lock, by holding the injected substance in place, contributes significantly to the effect exerted by Ca-EP.

Clinical studies comparing the efficacy of calcium electroporation and bleomycin-based electrochemotherapy

A double-blind, randomized, controlled trial was conducted at our department between October 2016 and June 2018 to compare the efficacy of Ca-EP and bleomycin-based ECT in skin metastases in 7 patients. This was the second clinical trial evaluating the efficacy of Ca-EP.

Patients included in our study were treated for at least one, and up to 10, histologically confirmed malignant melanoma or breast cancer metastases of 0.5-3 cm in size. After randomization, patients received either calcium or bleomycin intratumorally, followed by reversible EP. Six months after treatment, based on clinical and histological tumour response assessment, Ca-EP with bleomycin-based ECT achieved comparable efficacy to bleomycin-based ECT in eradicating tumours with a better side effect profile than ECT. CR after Ca-EP was confirmed by histological examination.

Seven patients with a total of 44 skin metastases (34 melanoma malignum, 10 breast cancer) were included in the clinical trial. Eleven metastases were taken for biopsies, 33 metastases were randomized and treated once. The OR was 33% (6/18) for Ca-EP and 53% (8/15) for bleomycin-based ECT, with overall response rates of 22% (4/18) and 40% (6/15), respectively. CR was confirmed by histopathological examination in both arms. No serious adverse events were recorded. CTCA grade I adverse events such as ulceration and hyperpigmentation were more frequent during bleomycin-based ECT than after Ca-EP. The effect of Ca-EP was "non-inferior" compared to bleomycin-based ECT. Our results suggest that Ca-EP is an effective and safe procedure for the treatment of cutaneous metastases located on the skin.

In the first clinical trial with Ca-EP in Denmark, 7 patients (6 breast cancer and 1 MM) were treated for skin metastases [62]. There was no significant difference in OR between Ca-EP, 72% (66% CR) and ECT 84% (68% CR). In this study, Ca-EP proved to be "non-inferior" and only mild side effects were observed with both treatment arms, such as ulceration of the treated area similar to those observed in our study (Table 7.).

The higher response rate in the first Danish study could be explained by the different histological type of the treated skin lesions and by the use of different electrodes. In our own study, we treated mainly malignant melanoma metastases (81.8%, n = 27), whereas in the Danish study the number of melanoma metastases was low (5.4%, n = 2).

In our clinical trial, 89% (24/27) of the treated melanoma metastases were BRAFV600E wild-type (WT). One study found bleomycin-based ECT to be more effective in melanoma showing BRAFV600E mutations compared with BRAF-WT tumours [69]. In both of the randomised clinical trials performed, this may explain the lower response rate than previous results, which requires further investigations on molecular level.

Another difference between the two clinical trials was the type of electrodes used. In our study, mainly (63.6%) hexagonal electrodes were used, whereas in the first study only linear electrodes were utilised. The electric field distribution is known to differ between linear (smaller diameter) and hexagonal electrodes. For this reason, the field distribution is more symmetric and less cold spots are observed with linear electrodes [70, 71]. Although no significant difference was observed due to the small number of cases, Ca-EP was more effective with linear electrodes ($p = 0.30$). In preclinical studies, increasing the electric field up to 0.8-1.0 kV/cm during Ca-EP significantly decreased ATP levels and the cell viability [35, 42]. This may explain the observed discrepancy, as the electric field was 1000 V/cm with linear and 910 V/cm with hexagonal electrodes. Further studies are needed to fully explore this issue.

There was no significant difference in the current delivered between the two treatment arms in any of the clinical trials. The difference may be more relevant in the treatment of large tumours. In our study, only grade I local adverse events were observed in both treatment arms. Both ulceration and hyperpigmentation occurred more frequently after bleomycin-based ECT (20% and 40%) than during Ca-EP (both 11%). Our observations were similar to the results of the first study: ulceration during Ca-EP affected only the tumour region, sparing the surrounding normal skin. None of the lesions treated with Ca-EP in the first study showed hyperpigmentation, which may be related to the exclusive use of linear electrodes.

Limitations of our study include the low number of metastases evaluated and the fact, that different electrodes (hexagonal or linear) were used for the treatments.

Adverse events of calcium electroporation

Ca-EP has a similar adverse event profile to bleomycin-based ECT, with the difference that the flu-like symptoms associated with chemotherapeutic agents do not occur with Ca-EP, and the treated skin rashes are less hyperpigmented. When the electrical pulses are delivered, there may be brief contractions in the muscles underlying the treated area. With local anaesthesia, muscle contractions may be uncomfortable. The treated area may show erythema, oedema and in some cases necrosis, which heals in 6-10 weeks. If necessary, painkillers or antibiotics could be used in the case of over-infection, in accordance with current guidelines.

The most commonly reported side effects of bleomycin-based ECT are post-operative pain (10%) and flu-like symptoms (10%). As the latter is due to bleomycin, this is not expected with Ca-EP. No CTCA stage 3-4 adverse events have been recorded [12]. Hyperpigmentation during bleomycin-based ECT occurred in 7% of cases [72], while after pooling the results of the first two clinical trials [62], it occurred in 5.55% of cases during Ca-EP (Table 7.).

5.2. Daylight photodynamic therapy in the treatment of actinic keratoses

AK was first described by Dubreuilh in 1826 [73, 74]. Clinically it presents on the UV exposed skin, and has different clinical forms (hypertrophic, atrophic, lichenoid, acantholytic and pigmented). Frequent localizations are the face and scalp, but AK can also occur on the back of the hands, forearms and shin. Usually it appears as a reddish-brown plaque on the skin with varying degrees of hyperkeratosis, and the severity is classified into three grades of (grade I-III). AK is often multiple and invades extensive areas of the skin surface, which is known as field cancerization. The diagnosis of AK is based on the clinical and dermoscopic findings, histological verification is performed only in certain cases [75].

Predisposing factors for the development of AK include cumulative UV exposure, fair skin type (Fitzpatrick I, II), advanced age, chronic exposure to arsenic or tar, immunosuppression (e.g. solid organ transplant recipients, hematology patients), human papilloma virus (HPV) infection and certain genodermatoses (e.g. xeroderma pigmentosum).

Although we can not predict which AK will progress towards invasive SCC, it is a fact, that 60-80% of invasive SCCs arise from AK. Therefore treatment of AK is of high importance, and PDT is a promising option [58, 59]. Compared to conventional PDT, daylight PDT is a simplified treatment procedure. In c-PDT, the photosensitizer is incubated on the skin for three hours in occlusion, while in d-PDT it is incubated for 30 minutes without occlusion. Another important difference is, that d-PDT uses the natural sunlight for the excitation of the photosensitizer, while c-PDT an artificial light source. Probably the major advantage compared to c-PDT is, that there is significantly less pain associated with d-PDT. The international protocol for d-PDT treatment is based on clinical trials conducted in centres mostly located in Northern Europe [46, 51, 52]. As d-PDT is performed in the open air and weather conditions may affect the feasibility, efficacy and tolerability of the treatment, it is necessary to adapt the protocol of the method to the local climatic conditions.

The aims of our work were to study the efficacy, safety and tolerability of d-PDT for the treatment of AK, and to introduce the intervention in our department. We used the international protocol of d-PDT and modified it step by step according to the results obtained during clinical trials in local climatic conditions [46, 51, 52].

We conducted three clinical trials with d-PDT. The average age of the patients in our clinical trials was around 75 years. We started to use the international protocol and based on tolerability, we modified first the the proportion of time patients spent indoors (from 30 to 120 min) and outdoors (from 90 to 30 min), while the overall length of treatment did not change significantly. Later, we introduced dosimetry to avoid high treatment doses. With our final protocol of d-PDT, the average treatment dose was around 20 J/cm² and the average treatment time was around 10 minutes. We found that performing d-PDT in AK with the modified protocol - optimizing the time spent outdoors and aiming for lower treatment doses - did not change the efficacy of the intervention, but was better tolerated by the elderly patient population.

We also compared results with using d-PDT to previous study results with c-PDT in our department, reported by Gaál *et al.* [45, 64]. We confirmed previus literature findings, that d-PDT was similarly effective but less painful compared with c-PDT.

Finally we have developed a local protocol for d-PDT in Szeged, and a description about the procedure for patients using pictures (Table 9., Figure 11.). Based on our protocol, the first step of d-PDT is to remove the hyperkeratosis from the skin area to be treated. A high factor sunscreen is recommended to be applied to the treatment area and to the surrounding skin. The treatment involves incubation of an extemporary formulated cream containing 10%

ALA on the skin for 120 minutes without occlusion. The upper threshold of the treatment dose is 100 J/cm^2, while taking into account the literature data, the lower threshold is 8 J/cm^2 [55]. In our studies, lesions treated with doses below and above 15 J/cm^2 showed no difference in clinical responses (CR and PR). Therefore, our we recommend to use lower treatment doses in d-PDT and avoid doses above 100 J/cm^2. To measure the actual light intensity, we recommend the use of dosimetry. From the light intensity and required treatment dose, it can be calculated how much time patients should spend outdoors. During summer time this time is recommended to keep around 10 minutes. Based ont the climatic conditions, we found it optimal in our region to carry out d-PDT between May and October. In rainy weather, and if the temperature outside is below 10 °C or above 35 °C, the treatment has to be postponed.

PDT was first used to treat cutaneous and subcutaneous tumours in 1978. The technique has evolved considerably since then, and innovation continues today. The use of d-PDT is hampered by certain weather conditions, which in some countries significantly limits its applicability [76, 77]. To overcome this problem, d-PDT has been tried in a greenhouse and indoors using light sources that simulate sunlight [78]. Wrist-mounted personal electronic dosimeters (wristwatch, SunSaver UV dosimeter) have been also developed to measure the light intensity required to determine the treatment dose. The importance of d-PDT for home use was already outlined and has been confirmed by the COVID19 pandemic. Treatment at home by the patient is facilitated by an app (SmartPDT) that can be downloaded to a smartphone [79]. The app determines the ideal day of treatment and the time to spend outdoors by satellite-based near real-time dosimetry measurements. Both the patient and the treating physician can monitor the treatment conditions and parameters using the app. It is also possible to upload a photo of the treated skin lesion, which allows the dermatologist to assess whether further treatment is necessary in the context of teledermatology.

6. CONCLUSIONS

The use of electrochemotherapy and photodynamic therapy in the treatment of skin tumours has undergone significant incremental innovations in the past decades. Two examples are the calcium electroporation and the daylight photodynamic therapy. Based on the results and new findings of our resent scientific work, both methods are promising to use in the routine dermato-oncological care.

6.1. Calcium electroporation in the treatment of cutaneous metastases

1. With Ca-EP similar efficacy was achieved in tumour eradication with a better adverse event profile than with bleomycin-based ECT. Our results confirmed the findings of the first clinical trial, that Ca-EP is "non-inferior" to bleomycin-based ECT in the treatment of cutaneous metastases [62].
2. Clinical complete remission after Ca-EP was confirmed for the first time by histological examination.
3. Given the growing number of cancers worldwide, Ca-EP may be a new, effective, and safe treatment for skin tumours, especially in patients for whom the use of chemotherapeutic agents are contraindicated.

6.2. Daylight photodynamic therapy in the treatment of actinic keratoses

1. We confirmed, that daylight PDT is effective in the treatment of AK under the local climatic conditions.
2. Daylight PDT in the treatment of AK is safe and well tolerated by our patients. We improved tolerability and safety of the method by reducing the time patient need to spend outdoor and by using dosimetry. The method is related with less pain compared to conventional PDT [64].
3. Daylight photodynamic therapy performed according to our modified version of the international treatment protocol was successfully introduced in our department.

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8. DECLARATION

Ethics approval of the study with calcium electroporation: The study protocol was approved and registered by the Centre for Health Registration and Education on 3 May 2016 under case number 032104/2016/OTIG. Approval and permission for our study was obtained from the Institutional and Regional Research Ethics Committee of Human Biomedical Sciences, University of Szeged (clinicaltrials.gov number: NCT03628417, registration date: 23 May 2016; permission number: 3806; registration number: 98/2016-SZTE; protocol number: ECT-KALCIUM-001).

Ethics approval of the study with daylight photodynamic therapy: Approval and permission for our study was obtained from the Institutional Review Board of the Albert Szent-Györgyi Medical Centre of the University of Szeged (registration date: 4 Nov 2014; registration number: 137/2014, protocol number: PDT-DLIGHT). (Institutional and Regional Research Ethics Committee of Human Biomedical Sciences, University of Szeged)

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10. SUPPLEMENT

Reprints of published papers (I-III)

I.

cancers

MDPI

Article

Evaluation of Calcium Electroporation for the Treatment of Cutaneous Metastases: A Double Blinded Randomised Controlled Phase II Trial

Dóra Ágoston [1], Eszter Baltás [1], Henriette Ócsai [1], Sándor Rátkai [1], Péter Gy Lázár [2], Irma Korom [1], Erika Varga [1], István Balázs Németh [1], Éva Dósa-Rácz Viharosné [1], Julie Gehl [3,4], Judit Oláh [5], Lajos Kemény [1] and Erika Gabriella Kis [1,*]

1 Department of Dermatology and Allergology, University of Szeged, 6720 Szeged, Hungary; agoston.dora@med.u-szeged.hu (D.Á.); baltas.eszter@med.u-szeged.hu (E.B.); ocsai.henriette@med.u-szeged.hu (H.Ó.); dr.ratkai.sandor@gmail.com (S.R.); korom.irma@med.u-szeged.hu (I.K.); varga.erika@med.u-szeged.hu (E.V.); nemeth.istvan.balazs@med.u-szeged.hu (I.B.N.); viharosne.eva@med.u-szeged.hu (É.D.-R.V.); kemeny.lajos@med.u-szeged.hu (L.K.)

2 Department of Oral and Maxillofacial Surgery, University of Szeged, 6720 Szeged, Hungary; lazarpgy@gmail.com

3 Center for Experimental Drug and Gene Electrotransfer (C*EDGE), Department of Clinical Oncology and Palliative Care, Zealand University Hospital, 4000 Roskilde, Denmark; kgeh@regionsjaelland.dk

4 Department of Clinical Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, 2200 Copenhagen, Denmark

5 Department of Oncotherapy, University of Szeged, 6720 Szeged, Hungary; lazarne.olah.judit@med.u-szeged.hu

* Correspondence: kis.erika.gabriella@med.u-szeged.hu; Tel.: +36-62-341-502

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Abstract: Calcium electroporation (Ca-EP) is a new anticancer treatment providing similar features to electrochemotherapy (ECT). The aim of our study is to compare the efficacy of Ca-EP with bleomycin-based ECT. This double-blinded randomized controlled phase II study was conducted at the Medical University of Szeged, Hungary. During this once only treatment up to ten measurable cutaneous metastases per patient were separately block randomized for intratumoral delivery of either calcium or bleomycin, which was followed by reversible electroporation. Tumour response was evaluated clinically and histologically six months after treatment. (ClinicalTrials.gov: NCT03628417, closed). Seven patients with 44 metastases (34 from malignant melanoma, 10 from breast cancer) were included in the study. Eleven metastases were taken for biopsies, and 33 metastases were randomised and treated once. The objective response rates were 33% (6/18) for Ca-EP and 53% (8/15) for bleomycin-based ECT, with 22% (4/18) and 40% (6/15) complete response rates, respectively. The CR was confirmed histologically in both arms. Serious adverse events were not registered. Ulceration and hyperpigmentation, both CTCA criteria grade I side effects, were observed more frequently after bleomycin-based ECT than for Ca-EP. Ca-EP was non-inferior to ECT, therefore, it should be considered as a feasible, effective and safe treatment option.

Keywords: calcium electroporation; bleomycin-based electrochemotherapy; cutaneous metastases; melanoma malignum; breast cancer; randomization; biopsy; non-inferiority

1. Introduction

Bleomycin-based ECT is a widely used method for the treatment of cutaneous tumours from all histologies [1–6]. During ECT, a chemotherapeutic drug, usually bleomycin, is electroporated into the

tumour cells, resulting in an increased cytotoxic effect. A recent meta-analysis of ECT in a palliative setting found a complete response (CR) rate of 46.6% and objective response rates (ORR) of 82.2%, regardless of the tumour type. Beyond its effectiveness, ECT is a repeatable and minimally invasive intervention that reduces symptom burden [7].

With the goal of reducing the risk of possible adverse events, the chemotherapeutic drug (bleomycin) is replaced with calcium in calcium electroporation (Ca-EP). Notably, calcium might be an effective option in cases where the administration of a chemotherapeutic drug is contraindicated. It was also expected that the use of Ca-EP will simplify the procedure and lower the treatment cost.

The mechanism of action and the anticancer efficacy of Ca-EP have been confirmed in preclinical studies. During Ca-EP treatment, electroporation increases intracellular calcium concentration, leading to increased ATP consumption. Additionally, the treatment leads to further loss of ATP production by opening permeability transition pores in mitochondrial membranes. This ATP depletion, together with other cellular effects, causes cell death [8].

The features of Ca-EP are very similar to ECT: they are both selective to tumour cell, the same pulsing conditions can be used for both procedures, and both have an anti-angiogenic effect in vitro and in vivo on both normal and tumour blood vessels [9,10]. It has also been demonstrated that Ca-EP initiates a systemic immune response. Thus, Ca-EP has the potential to replace bleomycin with calcium in electroporation treatments.

The first clinical trial of Ca-EP in humans was published recently [11]. The method was used with seven patients (six with breast cancer, one with malignant melanoma), and safety and non-inferiority in comparison with bleomycin-based ECT was proven.

To provide additional evidence on the efficacy and safety of Ca-EP, we decided to conduct a clinical trial. The aim of this study is to evaluate safety and efficacy of Ca-EP and to compare it with bleomycin-based ECT for cutaneous metastases.

2. Materials and Methods

This non-inferiority, phase II, double blinded, randomized confirmatory study investigated the efficacy of Ca-EP in comparison with the currently approved bleomycin-based ECT procedure for the treatment of cutaneous metastases. The study protocol was authorized by the Health Registration and Training Centre on 3 May 2016 and registered under the case number 032104/2016/OTIG. It was approved by the national and institutional ethical review boards (SZTE Regional Institutional Ethics Committee (23 May 2016, license no. 3806; registry no. 98/2016-SZTE). Patients provided written informed consent before enrolment.

The primary endpoint of the study was to compare the tumour response of cutaneous metastases after application of Ca-EP and ECT with the administration of intratumoral bleomycin. Tumour response was evaluated similar to response evaluation criteria in solid tumours (RECIST), v1.1, on each treated metastasis by clinical examination and digital photo documentation before and after treatment. The secondary endpoint was to evaluate and grade the toxicity of Ca-EP similar to Common Terminology Criteria for Adverse Events (CTCAE), version 4.0. The third endpoint was the measurement of the maximum safe and effective delivery of current to metastases using Ca-EP and bleomycin-based ECT.

Patients enrolled in the study had at least one histologically confirmed metastasis of 0.5 to 3 cm in size that was accessible to electroporation. A maximum of 10 cutaneous metastases per patient was included in the trial. Depending on the number of metastases present on the patient, one to six metastases were randomized into one of the two treatments and to the right or the left arm for evaluating response. Calcium was administered intratumorally to tumour(s) of one arm and bleomycin to the other and administration was immediately followed by electroporation of tumours on both arms.

Patient inclusion criteria were as follows: age >18 years, World Health Organization (WHO) performance status ≤2, life expectancy more than 3 months, platelet count ≥50 billion/L, international normalised ratio (INR) <1.5, and a period of more than 2 weeks of without treatment [12]. Only medical

cancer treatments (endocrine treatment, targeted treatment and radiotherapy to another area) were allowed. If there was no regression of cutaneous metastases, the continuation of vinorelbine, capecitabine or paclitaxel therapies were allowed. Patients were excluded from the trial if they had severe allergic reactions associated with bleomycin or if they previously received a dose of bleomycin that was more than 200,000 units/m^2. Pregnancy and lactation were also reasons for exclusion. Previously irradiated cutaneous metastases and concomitant treatments were recorded. The intervention was performed after oncological recommendation.

2.1. Randomization and Blinding

A clinical pharmacologist, who was independent of all the parties concerned with the study, was in charge of randomisation and kept the randomisation list secure, with the task of providing limited access only in case of emergency. The numbered metastases in each patient were block randomised 1:1 separately into the two treatment arms with the nQuery Adviser 7.0 computer program. The drugs to be given for each metastasis were prepared and labelled by a clinical pharmacologist. There was no need to cover the content of the ready syringes, as both bleomycin and calcium-chloride are colourless.

2.2. Procedure

The concentration of calcium chloride was estimated to be 220 mmol/L (9 mg/mL) from preclinical studies [9,12–14] and of bleomycin was 1000 IU/mL.

The volume to be injected was calculated according to the volume of the tumour. The drug volume for large tumours (>0.5 cm^3) was 0.5 mL/cm^3, while for small tumours (<0.5 cm^3) the volume was amended to 1 mL/cm^3. Tumour volume was calculated as ab2π/6, where a = longest diameter, b = longest diameter perpendicular to a.

Electric pulses were generated using a Cliniporator device (IGEA, Carpy, Italy) according to the standard operating procedures of the electrochemotherapy (ESOPE) guidelines. Linear needle electrodes (8 pulses of 400 V and 1000 V/cm, of 0.1 ms duration, at a frequency of 5 kHz) and hexagonal needle electrodes (4 pulses of 730 V and 910 V/cm, of 0.1 ms duration, at a frequency of 5 kHz) were used according to the tumour size and location. The anaesthesia during the procedure was either local or general, as planned during the consultation between the physician and the patient. Lidocaine with epinephrine was used as local anaesthetic and was injected in a square around the nodule. Changes in the response to anaesthesia were not observed.

Patients underwent the treatment once and were followed for 12 months with scheduled visits (7, 15, 30, 60, 90, 180 and 360 days after the treatment session).

At each follow up visit, the tumour response was evaluated clinically, and photos were taken. Response was categorised according to criteria similar to RECIST guidelines 1.1 as follows: complete response (CR), disappearance of all target lesions; partial response (PR), at least a 30% decrease in the sum of the longest diameter of target lesions; stabile disease (SD), neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for progression; and progression of the disease (PD), at least a 20% increase in the sum of the longest diameter of target lesions [15].

The randomization code was revealed 6 months after treatment and biopsies were taken from both calcium- and bleomycin-treated lesions. All biopsies were analysed by a histopathologist for the amount of tumour tissue, inflammation, fibrosis and necrosis.

Safety was evaluated with physical examinations and blood tests before and after treatment. Quality of life (QOL) score (0–100%) was also evaluated before and after the treatment. A numeric rating scale (NRS) (0–10) was used for assessing pain [16]. The Common Toxicity Criteria for Adverse Events, version 4.0, was used to register possible adverse events [17].

All Participants Assessed the Primary and Safety Analyses

The statistical analyses were performed using IBM SPSS, v24, software and R statistical program. Tumour response was analysed using Fisher's exact test on objective response 6 months after treatment,

Mann-Whitney test was used to measure the difference in delivered current between calcium-EP and bleomycin-based ECT, and the 2-sided 95% CI was used to measure the difference in outcome between the two groups [18]. For dimensioning the required number of cutaneous metastases, a non-inferiority study analysis was used: calculated with a significance level of 0.05 and a power of 80%, the results indicated that a minimum of 28 evaluable tumours were needed [19]. Taking into consideration the results of the previous clinical trial for the treatment of Ca-EP on cutaneous metastases as well as the basis of preclinical studies, a 20% non-inferiority margin was preset to detect a clinical difference between the two treatment arms.

3. Results

Seven patients (5 females, 2 males) with a total of 44 cutaneous metastases (34 from melanoma malignum and 10 from breast cancer) were enrolled in the clinical trial between October 2016 and June 2018 (Figure 1). Six patients had cutaneous metastases of malignant melanoma, localized on the lower extremity and one patient had metastases of breast cancer, localized on the trunk (Table 1). The patients median age was 73 years (Interquartile range: IQR = 21). Thirty-three metastases were randomized into the two treatment arms (left or right) of the study and were evaluated for clinical response, whereas 11 lesions were taken as biopsies (Figure 2). Eighteen of the randomized metastases were treated with Ca-EP (15 melanoma malignum cutan metastases, 3 breast cancer metastases) and 15 with bleomycin-based ECT (12 melanoma malignum cutan metastases, 3 breast cancer metastases). Six (18%) of the 33 randomized cutaneous metastases were located on a previously irradiated area (2 lesions were treated with Ca-EP, and 4 lesions with bleomycin-based ECT). According to the 33 response-evaluated metastases, the median was 7 mm (IQR = 5) of the largest diameters. The median injected volume for Ca-EP was 0.0855 mL (IQR = 0.1924), and 0.132 mL (IQR = 0.27) for bleomycin-based ECT (Table 2).

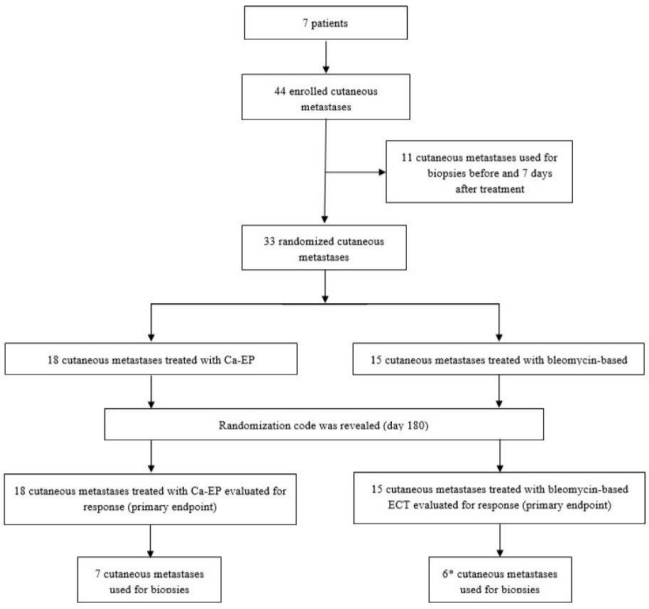

Figure 1. Trial profile. Illustration of trial profile. Further results are described in detail.

Table 1. Baseline demographic and clinical characteristics of the patients.

Patient	Sex Age (year)	Primary Tumour Characteristics	Location of Cutan Metastases	Number of Metastases Included/Evaluated	Years Since Diagnosis	Previous Therapy	Concomitant Treatment
1.	Male 76	pT3b, ALM, BRAF-WT	lower extr.	1/1	1	-	-
2.	Female 62	Breast: HER2-, ER+, PgR+	trunk	10/6	5.6	Epi-txt, Letrozole, mTORi	Letrozole
3.	Female 83	pT3b, NM, BRAF-WT, satellite met. Ing. sentinel: pos. BD: negative	lower extr.	9/6	7	adj. IFN, radiotherapy, ECT	-
4.	Female 49	pT3b, SSM	lower extr.	3/3	2	adj. IFN	-
5.	Female 83	pT3a, ALM, BRAF-WT Ing. sentinel: neg.	lower extr.	10/6	4.5	ECT	-
6.	Female 64	pT2a, SSM, BRAF-WT	lower extr.	6/6	2.75	-	-
7.	Male 73	pT3a, ALM, BRAF-WT	lower extr.	5/5	3.8	adj. IFN, radiotherapy	-
Total	5 Females, 2 Males mean: 70 (σ = 11.4891)	MM: 6, BRAF-WT: 5/6 Breast: 1	6 lower extr. 1 trunk	44/33	Mean 3.8 (σ = 1.9329)	various	

MM: malignant melanoma; ALM: acral lentiginous melanoma; extr.: extremity; NM: nodular melanoma; SSM: superficial spreading melanoma; BD: block dissection, BRAF WT: BRAF wild type; IFN: interferon; σ: standard deviation.

Table 2. Results of the current study in comparison with the study published in 2018.

Treatment Arm	Calcium-Electroporation			Bleomycin-Based Electrochemotherapy		
	Current Study	2018 Study	Total	Current Study	2018 Study	Total
Lesion characteristics						
Tumour size						
Median of the largest diameter, mm	6.5 (5–30)	9.5 (5–18)		7 (5–25)	11 (4–25)	
Tumour type						
Malignant melanoma	15	1	16	12	1	13
Breast cancer	3	17	20	3	18	21
Previously irradiated lesions, *n*	2	8	10	4	7	11
Location						
Lower extremity	15	4	19	12	4	16
Trunk	3	14	17	0	15	15
Upper extremity	0	0	0	3	0	3
Treatment						
Median doses (range), mL	0.085 (0.042–3.14)	0.24 (0.03–1.21)		0.132 (0.065–0.475)	0.21 (0.03–0.55)	
Median delivered current (range), A	3.85 (1.4–9)	3.4 (0.9–8.2)		4 (1.4–6.5)	2.8 (1–9.6)	
Median delivered current with linear electrodes (range), A	4 (2.25–9)	3.4 (0.9–8.3)		*5.05 (4–6.1)*	2.8 (1–9.6)	
Median delivered current with hexagonal electrodes (range), A	2.5 (1.4–4.2)	NA		*2.75 (1.4–3.6)*	NA	
Median number of applications (range), *n*	1 (1–6)	3 (1–7)		1 (1–3)	3 (1–7)	
Electrodes						
Linear	39% (7)	100% (18)		33% (5)	100% (19)	
Response (CR) for linear electrode subgroup	14% (1)	66% (12)		0	68% (13)	

Table 2. *Cont.*

Treatment Arm	Calcium-Electroporation			Bleomycin-Based Electrochemotherapy		
	Current Study	**2018 Study**	**Total**	**Current Study**	**2018 Study**	**Total**
Lesion characteristics						
Hexagonal	61% (11)	0		67% (10)	0	
Response (CR) for hexagonal electrodes subgroup	27% (3)	NA		60% (6)	NA	
Response						
Complete response, percent (*n*)	4	12	44.44% (16)	6	13	55.88% (19)
Partial response, percent (*n*)	2	1	8.33% (3)	2	3	14.7% (5)
Stable disease, percent (*n*)	6	3	25% (9)	5	0	14.7% (5)
Progressive disease, percent (*n*)	6	2	22.22% (8)	2	3	14.7% (5)
Adverse events						
Ulceration, percent (*n*)	2	7	25% (9)	3	13	47.05% (16)
Itch, percent (*n*)	0	1	2.77% (1)	0	5	14.7% (5)
Hyperpigmentation, percent (*n*)	2	0	5.55% (2)	6	5	32.35% (11)
Exuding, percent (*n*)	0	2	5.55% (2)	0	2	5.88% (2)

Current study: Evaluation of calcium electroporation for the treatment of cutaneous metastases; a double blinded randomized controlled phase II trial, Department of Dermatology and Allergology, University of Szeged (ClinicalTrials.gov: NCT03628417). 2018 study: Calcium electroporation for treatment of cutaneous metastases; a randomized double-blinded phase II study, comparing the effect of calcium electroporation with electrochemotherapy, Denmark [11].

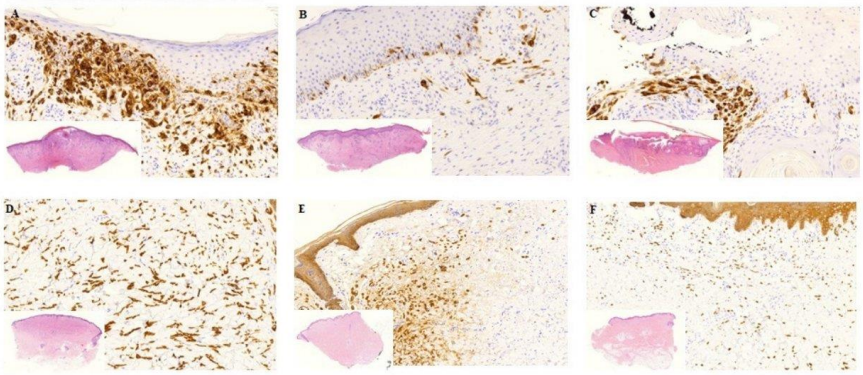

Figure 2. Histology from biopsies before and 7 days after treatment. Biopsies before and one week after treatment. (**A**–**C**) Patient nr. 5 with MM. A: Pre-treatment biopsy: Extensive tumour infiltration, mild fibrosis, moderate lymphocytic inflammation, no necrosis. The tumour cells show diffuse MelanA positivity. (**B**) Day 7. Post-treatment with Ca-EP: Partly ulcerated skin, moderate tumour infiltration and fibrosis, mild inflammation, no necrosis. Only scattered MelanA positive tumour cells. (**C**) Day 7. Post treatment with bleomycin-based ECT: Partly fragmented, ulcerated skin with pseudoepitheliomatous hyperplasia of the epidermis, moderate fibrosis and inflammation, no necrosis. Focal MM nests with MelanA positivity. (**D**–**F**) Patient nr. 2 with breast cancer. (**D**) Pre-treatment biopsy: Extensive breast cancer infiltration without fibrosis, inflammation and necrosis. The tumour cells are CKAE1/AE3 positive. (**E**) Day 7. Post-treatment with Ca-EP: Focal tumour infiltration, very mild inflammation no fibrosis or necrosis. (**F**) Day 7. Post treatment with bleomycin-based ECT: Dispersed tumour cells with CKAE1/AE3 positivity, mild inflammation, no fibrosis or necrosis. MM: malignant melanoma, CK: cytokeratin. Histological photos: digital scanning with magnification approximately 5–20×.

Four of the procedures were conducted under local, and three of them under general anaesthesia. Hexagonal needle electrodes were used for electroporation of 21 metastases, (63.6%), whereas linear electrodes were used for 12 lesions (36.4%). Of the 18 lesions receiving Ca-EP, 11 were treated with hexagonal (61.1%) and 7 with linear electrodes (38.9%), respectively. Of the 15 lesions receiving bleomycin-based ECT, 10 were treated with hexagonal electrodes (66.7%), and 5 with linear electrodes (33.3%).

3.1. Tumor Response

The ORR for Ca-EP was 33% (CR = 22%; PR = 11%) and for bleomycin-based ECT was 53% (CR = 40%; PR = 13%) (Figure 3). The difference was not significant neither in OR ($p = 0.30$) nor in CR ($p = 0.45$) between Ca-EP and bleomycin-based ECT. After 6 months, 33% (6 of 18) metastases treated with calcium, and 13% (2 of 15) metastases treated with bleomycin had progressed. The two-sided 95% CI for the outcome difference between the two groups was −13.3%–53.3%. There was no significant difference in response between previously irradiated and non-irradiated lesions ($p = 0.37$). Six months after treatment, the randomization code was revealed, and 6 biopsies (3 from Ca-EP, 3 from bleomycin-treated lesions) taken from the 13 tumours exhibited a clinically CR. For 5 of these 6 biopsies, a CR was confirmed by histology (Figure 4). No tumour cells were identified in the 3 lesions treated with Ca-EP and in 2 of the lesions treated with bleomycin-based ECT.

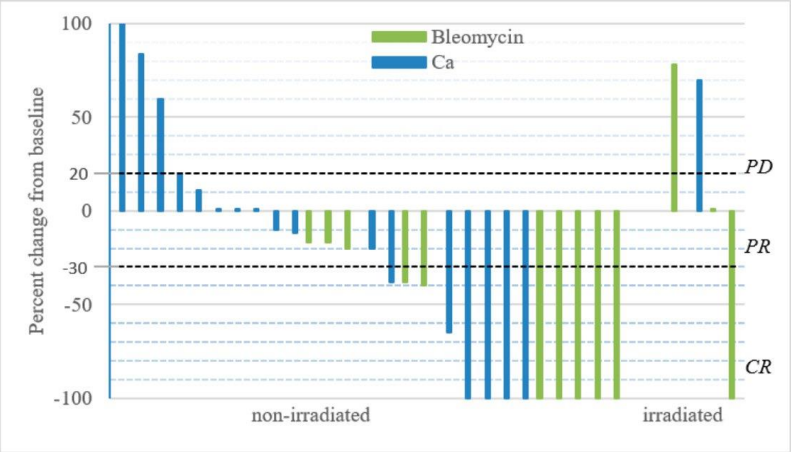

Figure 3. Change in tumour size over time. Metastases were treated at day = 0 with either i.t. calcium and or i.t. bleomycin in a randomized double-blinded study design. Patients received only one treatment and response was evaluated 6 months after treatment, after the randomization code was revealed. Change in size over time; the graph illustrates the percent change in tumour size recorded 6 months after treatment. The two non-measurable metastases treated with calcium-chloride and bleomycin were irradiated, and are not included in the graph, but were included in the response analysis as PD.

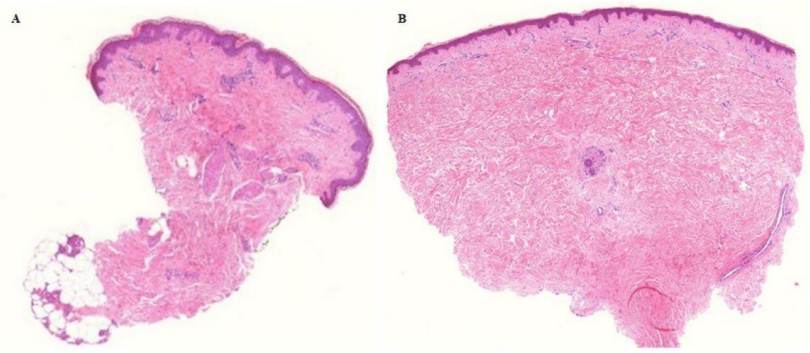

Figure 4. Histologically confirmed complete remission 6 months after Ca-EP. Tumour cells were not identified 6 months after Ca-EP neither in malignant melanoma (**A**) nor in breast cancer metastases (**B**). A: patient no 3, Ca-EP treated clinically CR melanoma malignum cutaneous metastasis. B: patient no 2, Ca-EP treated clinically CR breast cancer cutaneous metastasis. Histological photos: digital scanning with low magnification.

Regarding tumour response and the type of electrode, we observed differences between the two treatment arms that because of the small sample size was statistically not significant. The ORRs obtained with Ca-EP cutaneous metastases were higher for linear electrodes (42.8%, 3/7) than for hexagonal electrodes (27.3%, 3/11) ($p = 0.63$). Of the tumours that were categorized as PD, 45.45% (5/11) were treated with hexagonal and 14.29% (1/7) with linear electrodes ($p = 0.32$). The opposite trend was observed with bleomycin-based ECT. The ORR was 70% (7/10) with hexagonal and 20% (1/5) with

linear electrodes (p = 0.12). Of the tumours that were categorized as PD, 10% (1/10) were treated with hexagonal and 20% (1/5) with linear electrodes (p = 1).

Six patients had long-term follow-up over a mean of 29 months (standard deviation: σ = 6.8232). One patient died 11 months after the study treatment session because of the progression of another primary tumour and two additional patients died 26 and 27 months after treatment because of a hip fracture and progression of the melanoma, respectively. None of the cutaneous metastases categorized as CR relapsed during the 1-year follow-up period.

3.2. Adverse Events

Serious adverse events were not observed. Ulceration and hyperpigmentation, both CTCA criteria grade I side effects, were seen after Ca-EP in two metastases each (2/18, 11%). After bleomycin-based ECT, ulceration was observed in 20% (3/15) and hyperpigmentation in 40% of the treated lesions (6/15) (Figure 5). The median NRS score before treatment was 2 (IQR = 2). Immediately after treatment the median NRS score was 2 (IQR = 9), 3 patients reported no pain (NRS: 0), 1 patient reported mild pain (NRS: 1–3) and 1 patient moderate pain (NRS: 4–6). Two patients, both having more than six metastases and who underwent biopsies, reported severe pain (NRS: 9–10). Six months after treatment the median NRS score was 2 (IQR = 4), with medium values of 2.5 (σ = 3.2016) for patients treated with Ca-EP and 4.5 (σ = 2.2913) for patients treated with ECT.

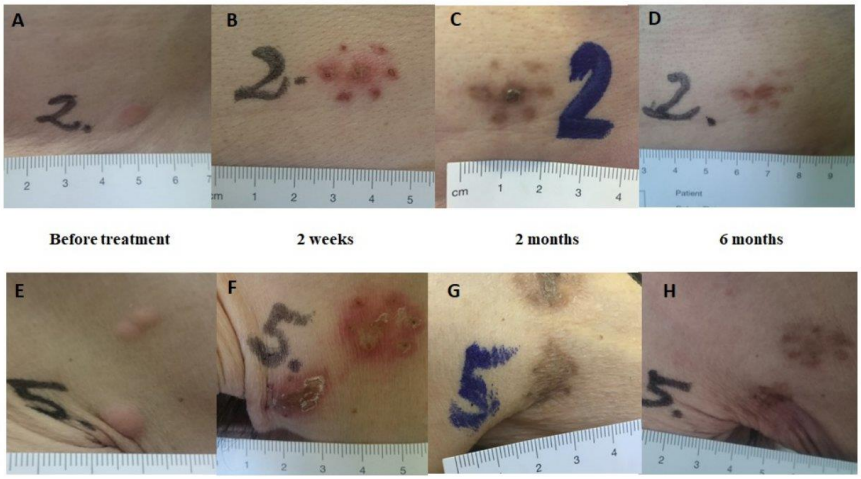

Figure 5. Clinical response. Clinical response after Ca-EP; clinical response after bleomycin-based ECT. The lesions are from patient no.2 with cutaneous metastases from breast cancer in the same region (trunk). Lesion no. 2: Ca-EP treated cutaneous metastasis. Lesion no. 5: bleomycin-based ECT treated cutaneous metastasis. (**A**,**E**) Before treatment. (**B**,**F**) Two weeks after treatment; typical crust appearance. (**C**,**G**) Two months after treatment; clear hyperpigmentation in the areas treated with calcium and bleomycin. (**D**,**H**) Six months after treatment; complete disappearance of metastases.

Six months after treatment, the QOL scores were equal to or higher than before treatment. The median QOL score was 70% (IQR = 10) before treatment and 80% (IQR = 10) at day 180. Three patients did not report a change in their quality of life during the 6 months after treatment. All 7 patients reported they would agree to repeat the treatment if necessary.

3.3. Delivered Current

There was no significant difference in the measured delivered current neither between the two treatment arms ($p = 0.956$) nor in non-irradiated metastases compared to irradiated metastases ($p = 0.911$). The median delivered current was 3.85 A (IQR = 3.75) in metastases treated with calcium and 4 A (IQR = 2.4375) with bleomycin. The median delivered current in metastases located in non-irradiated skin was 3.85 A (IQR = 3.45) and 3.95 A (IQR = 1.525) in metastases from previously irradiated skin. A total of 30 applications were used with a median value of 1 (range 1–6, IQR = 1) to the 18 randomized lesions treated with Ca-EP, and 22 applications with a median value of 1 (range 1–3, IQR = 1) to the 15 cutaneous metastases treated with bleomycin-based ECT. The median delivered current measured with linear electrodes in the two treatment arms (Ca-EP and bleomycin-based ECT) was 5 A (range 2.25–6.1, IQR = 2.1; 31 applications) and 2.5 A (range 1.4–4.2, IQR = 1.6; 21 applications) with hexagonal electrodes. In the Ca-EP arm the median delivered current was 2·5 A (range 1.4–4.2, IQR = 1.5; 11 applications) with hexagonal and 4 A (range 2.25–9, IQR = 3.5; 19 applications) with linear electrodes. In the bleomycin-based ECT group the median delivered current was 2.75 A (range 1.4–3.6, IQR = 1.7125; 10 applications) with hexagonal and 5.05 (range 4–6.1, IQR = 1.325; 12 applications) with linear electrodes (Table 2).

3.4. Discussion

Ca-EP is a novel anticancer treatment that has been used successfully with tumours exhibiting various histologies. Preclinical studies provided the first support for the efficacy across cancer histologies, as well as an explanation of the mechanisms of action [11,13,14,20–27]. The results of the first clinical trials suggested that Ca-EP is safe and efficient at the local level for tumours of different types, including cutaneous metastases from breast cancer and malignant melanoma and recurrent head and neck cancer [11,20]. Moreover, a case report showed that Ca-EP is able to initiate a systemic immune response and target untreated metastases in a patient suffering from malignant melanoma [28]. In the Ca-EP procedure, no chemotherapeutic drug is administered to the patient, and, therefore it is ideal in cases when chemotherapy is contradicted (e.g., severe lung function impairment, pregnancy etc.). Ca-EP is a simple and inexpensive cancer treatment and can lead to good cosmetic outcome.

We were encouraged to implement the second trial with Ca-EP on cutaneous metastases by the results of the first clinical studies and by the possibility that Ca-EP can be used in cases when bleomycin cannot be administered.

In our study, which included six patients with malignant melanoma and one patient with breast cancer, we demonstrated that Ca-EP is safe and effective in the treatment of small cutaneous metastases. Importantly, the CR seen clinically after Ca-EP was confirmed in our study by histology. The ORR for Ca-EP was lower (ORR = 33%, CR = 22%) than for bleomycin-based ECT (ORR = 53%, CR = 40%), but the differences were not significant for OR ($p = 0.30$) and for CR ($p = 0.45$). Our preset criteria for the non-inferiority for Ca-EP was proven. The first clinical trial performed in Denmark included seven patients: six patients with cutaneous metastases of breast cancer and one patient with malignant melanoma [11]. The results from this study had a similar tendency toward higher ORR and no significant difference between Ca-EP, 72% (with 66% CR), and ECT, 84% (with 68% CR). The first trial also proved non-inferiority for Ca-EP, and only mild adverse events were observed for both treatments, including ulcers in the treated area, similarly to our study. The higher response rates achieved by the Danish study could be explained by the different histotypes of the treated cutaneous metastases and also with the use of different electrodes. We have treated mainly malignant melanoma metastases (81.8%, $n = 27$), whereas the number of melanoma metastases was small (5.4%, $n = 2$) in the Danish study. The primary tumour characteristics of our study show that 89% (24/27) of the response evaluated melanoma metastases were BRAF-WT (wild-type or non-mutated). A recent study revealed that bleomycin-based ECT is more effective on BRAF mutated malignant melanoma cells in comparison with WT (non-mutated) melanoma cells [29]. These results could account for the lower response rates on both arms of the current study and require further investigation.

The other difference between the two trials were the electrodes used; only linear electrodes were used in the Danish, where as our study used mainly (63.6%) hexagonal electrodes. It is known that the electric field distribution is different between the linear (which have a smaller diameter) and hexagonal electrodes. Therefore, with linear electrodes field distribution is much more symmetrical with less cold spots. [30,31] Although a significant difference was not detected because of the small number of events, Ca-EP was more effective in our trial with linear electrodes, ($p = 0.30$). In preclinical studies significantly decreased ATP level and cell viability were observed by increasing the electric field from 0.8 to 1.0 kV/cm during Ca-EP [9,23]. These findings could explain the observed difference as the electric field was 1000 V/cm with the use of linear and 910 V/cm with hexagonal electrodes. Because of the small sample size of the current trial, further studies are needed to fully uncover the question.

There was no significant difference in the measured delivered current between the two treatment arms in the two finished studies. The difference in conductivity may be more relevant when treating large tumours.

Only grade I local adverse events were seen in both treatment arms in our study. Both ulceration and hyperpigmentation were observed more often after bleomycin-based ECT (20% and 40%) than after Ca-EP (both 11%). Our observations of Ca-EP skin toxicity were similar to those of the previous trial: only the tumour region was affected by the ulcer, whereas the surrounding normal skin was spared. In the Danish trial, none of the lesions treated with Ca-EP exhibited altered cutaneous pigmentation, which might be related to the exclusive use of linear electrodes.

The limitations of our study include a small number of enrolled metastases and the use of different electrodes.

4. Conclusions

Ca-EP proved to be safe and effective in eradicating tumours, and this conclusion was confirmed histologically. Our results are in agreement with the results of the first clinical trial on Ca-EP, which showed that Ca-EP was non-inferior to bleomycin-based ECT, and therefore Ca-EP should be considered as a feasible treatment for patients with cutaneous metastases for which other chemotherapeutic drugs are contraindicated.

The configuration of the electrode and the histotype may influence tumour response. Further studies are needed to establish a strong evidence base in the treatment of cutaneous metastases with Ca-EP.

Author Contributions: Conceptualization, E.G.K., S.R. and D.Á.; methodology, J.G.; validation, E.G.K., S.R. and D.Á.; formal analysis, É.D.-R.V.; investigation, E.G.K., S.R., D.Á., P.G.L., E.B., H.Ó., J.O., E.V., I.K., I.B.N. and L.K.; data curation, E.G.K., S.R. and D.Á.; writing—original draft preparation, D.Á.; writing—review and editing, E.G.K.; visualization, E.G.K., E.B. and D.Á.; supervision, E.G.K.; project administration, E.G.K. All authors have read and agreed to the published version of the manuscript.

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II.

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A Merkel sejtes karcinóma multimodális kezelési lehetőségei

Multimodal treatment options for Merkel cell carcinoma

ÁGOSTON DÓRA DR.[1], HÁNIS CSILLA DR.[1], ÓCSAI HENRIETTE DR.[1,2], CSÁNYI ILDIKÓ DR.[1], VARGA ERIKA DR.[1], KOROM IRMA DR.[1], NÉMETH ISTVÁN DR.[1], KIS ERIKA DR.[1], KEMÉNY LAJOS DR.[1], OLÁH JUDIT DR.[1,3], BALTÁS ESZTER DR.[1]

Szegedi Tudományegyetem, Általános Orvostudományi Kar,
Szent-Györgyi Albert Klinikai Központ, Bőrgyógyászati és Allergológiai Klinika[1]
Békés Megyei Központi Kórház, Pándy Kálmán Tagkórház, Gyula[2]
Szegedi Tudományegyetem, Általános Orvostudományi Kar,
Szent-Györgyi Albert Klinikai Központ, Onkoterápiás Klinika[3]

ÖSSZEFOGLALÁS

A Merkel sejtes karcinóma egy ritka, agresszív viselkedésű, neuroendokrin bőrtumor. Patogeneziséből a Merkel sejt poliómavírus klonális integrációja és az UV fény emelhető ki. Kialakulásának rizikótényezői az előrehaladott életkor, a krónikus UV expozíció, a kaukázusi bőrtípus, a férfi nem és az immunszuppresszió. A betegség gyors progresszióra hajlamos és a prognózisa kedvezőtlen. Kezelésében elsődleges a primer tumor sebészi eltávolítása és az őrszem nyirokcsomó biopszia, illetve kiemelt jelentőségű az adjuváns sugárterápia. A szisztémás kezelések közül első vonalban immunellenőrzőpont-gátlók javasoltak. A szerzők a diagnosztikára és a kezelésre vonatkozó legújabb európai irányelvet ismertetik.

Kulcsszavak:
Merkel sejtes karcinóma – Avelumab – őrszem nyirokcsomó biopszia

SUMMARY

Merkel cell carcinoma is a rare neuroendocrine skin tumor with an aggressive behavior. The clonal integration of the Merkel cell polyomavirus and the UV light can be highlighted in its pathogenesis. Risk factors for its development are advanced age, chronic UV exposure, Caucasian skin type, male gender and immunosuppression. The disease is prone to rapid progression and the prognosis is unfavorable. In its treatment, surgical removal of the primary tumor and biopsy of the sentinel lymph node, as well as adjuvant radiation therapy, are of particular importance. Among the systemic treatments, immune checkpoint inhibitors are recommended as first line. The authors discuss the latest European guideline for the diagnosis and the treatment.

Key words:
Merkel cell carcinoma – Avelumab – sentinel lymph node biopsy

Definíció, epidemiológia

A Merkel sejtes karcinóma (MCC) egy ritka, agresszív viselkedésű, neuroendokrin bőrtumor. Elsőként az irodalomban ötven évvel ezelőtt (1972) Cyril Toker a tumor verejtékmirigy eredetét feltételezve a bőr trabekuláris karcinómájaként említi (1). A tumor neuroendokrin eredetét 1978-ban Tang és Toker bizonyította be, a neuronspecifikus enoláz (NSE) jelenlétét kimutatva (2). Jelenlegi elnevezését 1980-ban kapta, mely terminológia a tumorsejtek és a bőr bazális rétegében lévő Merkel sejtek közötti hasonlóságra utal (3). A pontos kiindulási sejttípus napjainkig ismeretlen, epidermális őssejtek, fibroblasztok vagy korai B-sejtek merültek fel (4).

Az MCC a malignus bőrdaganatok kevesebb mint 1%-át alkotja, a melanoma malignumnál kb. ötvenszer ritkábban fordul elő (5). Az MCC incidenciája az elmúlt évtizedben jelentősen emelkedett, mely tendencia folytatódása várható világszerte. Egy 2017-es összefoglaló közleményben tíz ország (Európa, Ausztrália, Új-Zéland) adatait elemezték, mely alapján az incidencia 0,1-1,6 közé tehető 100 000 életévre vonatkoztatva (5). A legmagasabb érték Ausztráliában adódott, melyet részben a magas ultraibolya (UV) fény expozícióval magyaráztak. Az Egyesült Államokban 2000 és 2013 között előfordulása csaknem megduplázódott. Az MCC emelkedő előfordulásáért legfőképpen a tumoros betegség valódi incidencia növekedése tehető felelőssé, azonban a tumor regiszterek tökéletesedése, az immunhisztokémiai vizsgálómódszerek fejlődése, a virális karcinogenezis szerepének felfedezése, valamint a gyakorló orvosok elméleti és gyakorlati ismereteinek bővülése is hozzájárult (5).

Levelező szerző: Ágoston Dóra dr.
e-mail: agostondora@yahoo.com

A betegség patogenezise

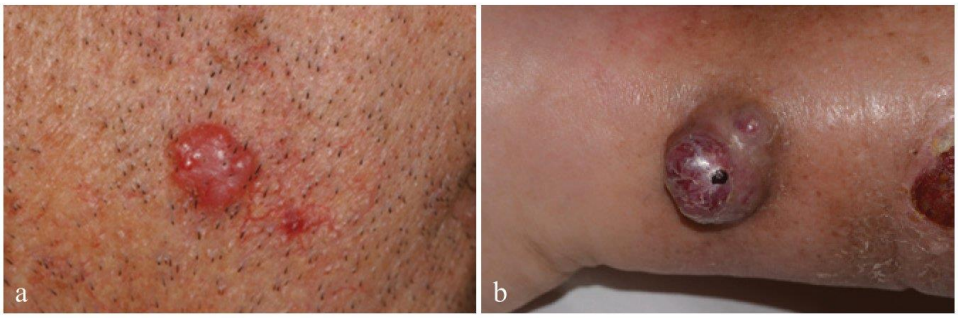
Az MCC patogenezisében a Merkel sejt poliómavírus (MCPyV), az UV fény expozíció és az immunszuppresszió emelhetőek ki. A tumor patogenezisének tanulmányozásában nagy áttörést jelentett 2008-ban a Feng és mtsai által izolált DNS-poliómavírus (MCPyV) kimutatása, melyet a későbbiekben több munkacsoport is megerősített (6, 7). A MCPyV a humán bőr mikrobiom része. A virális DNS klonálisan integrálódik a sejtek genetikai állományába, mely véletlenszerűen számos helyen történhet. Martel-Jantin *és mtsai.* leggyakrabban (21%) az 5-ös kromoszómán mutatták ki jelenlétét, illetve ennél kisebb arányban (5–16%) további nyolc (1., 3., 4., 6., 11., 14., 18., 19.) kromoszómán. A tumor kialakulásában résztvevő gazdasejt gén ismeretlen. A MCPyV kettős szálú cirkuláris DNS-ének felszakadása leggyakrabban (70%) a genomjának több mint felét alkotó „large T" (LT) régióban következik be. A virális LT-fehérje által hordozott konzervált szakaszok (pl.: DnaJ, pp2A-kötő domének) onkogén hatása poliómavírusokban bizonyított (pl.: SV40). Feltételezhetően külső hatásra a tumorgenomba integrált virális LT-antigén helikáz régiója károsodik, így bár a vírus fertőző képessége megszűnik, a károsodott onkoprotein a daganat további fejlődésében játszik szerepet (8). A daganat fenntartásában az apoptózist gátló BIRC5a (baculoviral inhibitor of apoptosis repeat-containing 5a) onkoprotein vesz részt. (9). Irodalmi adatok szerint a vaszkuláris endoteliális növekedési faktor receptor-2 (VEGFR-2) expresszió összefüggést mutat a daganat méretével és metasztatizáló képességével. A tumor kialakulásában a mammalian target of rapamycin (mTor) receptorok, valamint az AKT/PI3C és Ras jelátviteli útvonalak szerepét is feltételezik. Az MCC pathogenezisében az UV sugárzásnak is jelentős szerepet tulajdonítanak, melyet az epidemiológiai adatok is alátámasztanak.

Jelen ismereteink alapján megkülönböztetünk MCPyV pozitív (80%) és MCPyV negatív (20%) vagy UV sugárzás indukálta tumorokat. A közelmúltban végzett genetikai elemzések sokkal magasabb UV sugárzás indukálta mutációs terhelést (p53, TP53, RB1, NOTCH1, HRAS) találtak a MCPyV negatív daganatokban. Megállapították, hogy az immunválaszt a citozin-timin (C-T) mutációk magasabb száma és az ennek következtében kialakuló új tumor-aszszociált epitopók váltják ki (10). A MCPyV pozitív MCC-t alacsony frekvenciájú szomatikus mutációk jellemzik. Döntő többségük jól reagál immunonkológiai kezelésre, mely során a T-sejtek a vírusok által termelt onkoproteineket ismerik fel (11, 12, 13, 14). Egyértelmű adatok nincsenek arra vonatkozólag, hogy a virális státusz hogyan befolyásolja a prognózist. A vírus pozitív tumorok jobb prognózisúak, azonban immunonkoterápiára adott válasz tekintetében szignifikáns különbség a MCPyV pozitív és negatív tumorok között nincsen (15).

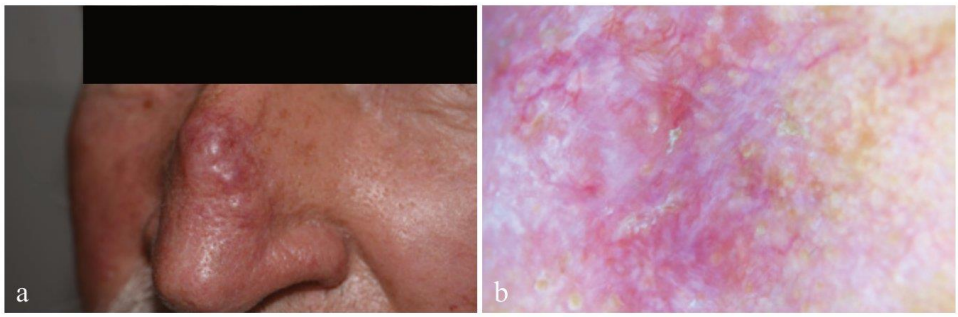
Klinikai megjelenés, rizikótényezők, diagnózis

Az MCC kialakulásának rizikótényezői az előrehaladott életkor (75-80 év), a krónikus UV expozíció, a kaukázusi bőrtípus (az esetek 95%-a), a férfi nem (férfi:nő=2,5:1) és az immunszuppresszió (hematológiai betegség, HIV-fertőzés, szerv transzplantáció). Az MCC 6-12%-ban jelentkezik immunszupprimált betegeknél. Az MCC megjelenésének kockázata tízszeres a szervtranszplantáltak között, tizenháromszoros a HIV betegek körében, míg 30-50-szer gyakrabban fordul elő hematológiai alapbetegség esetén (16, 17, 18). Immunszuppresszió esetén az MCC kialakulása fiatalabb életkorra tehető, a betegség lefolyása kedvezőtlenebb és mortalitása magasabb.

Az MCC klinikai jellegzetességeit klinikánkon 25 Merkel sejtes karcinómával diagnosztizált beteg esetében tanulmányoztuk, rektrospektív vizsgálat keretén belül 2000-2016 között. Vizsgálatunk alapján az MCC klinikailag általában szubjektív panaszt nem okozó, leggyakrabban bőrszínű vagy pink papula vagy előemelkedő tumor volt, mely rapidan növekedett *(1. a, b ábra)*. Vizsgálatunkban a tumorok átlagos átmérője 18 mm volt (3-45 mm), vérzés és kifekélyesedés 20%-ban fordult elő. Irodalmi adatok alapján az esetek 5%-ában a primer tumor ismeretlen és a betegség a nyirokcsomókból indul ki. Vizsgálatunkban egy beteg esetében volt a primer tumor ismeretlen. Nyálkahártyáról való kiindulást, mely irodalmi ritkaságnak számít, nem tapasztaltunk (19). Betegeink átlagos életkora 72 év (42-89 év) volt, mely a világirodalomban közölt adatokkal korrelál, míg a nemek aránya tekintetében eltérést tapasztaltunk. Betegeinknél a nemek aránya megegye-



1. a, b ábra
Primer Merkel sejtes carcinoma az arcon *(a)* és az alsó végtagon *(b)*



2. a, b ábra

Recidíváló Merkel sejtes carcinoma klinikai és dermatoszkópos képe krónikus limfoid leukémiában szenvedő betegnél
a: Recidív tumor az orrháton, *b:* Vörös és fehér homogén, strukúramentes területek, polimorf erek, fehér vonalak

ző volt, míg általában férfi predominancia észlelhető. Az MCC leggyakrabban a fej-nyak régióban (50%) fordul elő, melyet a végtagok (40%) és a törzs (10%) követnek. A vírus pozitív és negatív tumorok lokalizációjában lényeges különbség jelenlegi ismereteink alapján nincsen.

Vizsgálatunkban a primer tumor lokalizációját tekintve a daganatok 40%-a az arc területén, 32% a felső, 20% az alsó végtagon és 8% a törzsön jelent meg.

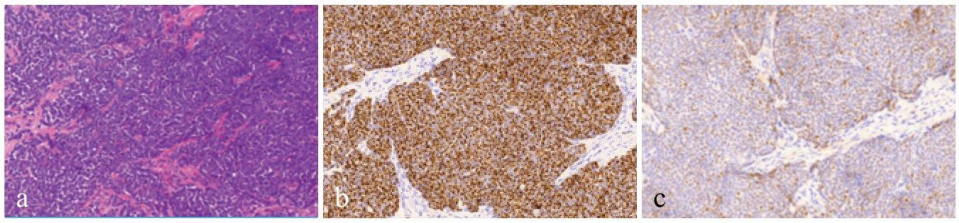
A differenciál diagnosztikában elsősorban benignus (hemangióma, lipóma, plazmocitóma, ateróma, epidermális ciszta, dermatofibróma) és malignus bőrdaganatoktól (bazálsejtes karcinóma, bőr laphámrák, amelanotikus melanoma, adnexális tumor, dermatofibróma, kután áttét, kután limfóma) kell elkülöníteni.

Vizsgálatunkban a betegek általában a tumor méretbeli növekedése miatt fordultak orvoshoz. Az általunk vizsgált 25 esetből 23-ban találtunk az ambuláns lapokon információt arra vonatkozólag, hogy mennyi idő telt el a primer tumor észlelése és a szövettani diagnózis felállítása között. Ez az idő átlagosan 6 hónap volt (1-48 hónap), mely jóval alacsonyabb a melanomás betegeinknél tapasztaltakhoz képest (18 hónap) (20). A különbség véleményünk szerint az MCC gyors progressziójával és az érintett betegpopuláció magas életkorával hozható összefüggésbe.

Az MCC helyes diagnózisa sok esetben késik. A főbb klinikai jellegzetességek alapján összeállított mozaikszó segítségül szolgálhat a gyakorló orvosoknak a helyes klinikai diagnózis felállításában (AEIOU, A: asymptomatic, E: expanding rapidly, I: immunosuppression, O: older than 50 years of age, U: UV exposed site).

Az MCC dermatoszkópos képe nem specifikus *(2. a, b ábra).* Legfőbb jellemzői egyéb malignus tumorokban (pl. előrehaladott bőr laphámrák, amelanotikus melanoma) is előfordulhatnak. Pink, fehér vagy milky-red homogén stuktúramentes területek és polimorf erek (pontszerű, rövid lineáris, kanyargós) jellemzik, de előfordulhat ulceráció és fénylő fehér vonalak.

Az MCC-nek szövettanilag három formája ismert: intermedier, kissejtes és trabekuláris típus, az esetek nagy részében azonban kevert típussal találkozunk (21). A szövettani képre a kis bazofil, kerek sejtek csoportjai, a sejtek finom kromatinstruktúrája, a nukleolusz hiánya, valamint számos mitotikus alak jellemző *(3. a ábra).* A tumorsejtek a bőr normál Merkel sejteihez hasonlóan sűrűn elhelyezkedő perinukleáris neuroszekréciós granulomokat tartalmaznak. Immunhisztokémiailag MCC-ben diagnosztikus értékű a perinukleáris, pontszerű CK20 pozitivitás és a kissejtes tüdőrákra jellegzetes TTF1 (pajzsmirigy transzkripciós faktor 1) negativitás *(3. b ábra).* Bizonyos esetben neuroendokrin markerek (neuronspecifikus enoláz, kromogranin A/B, szinaptofizin) és a MCPyV T antigén is kimutatható *(3. c ábra).* A melanomától és a limfómáktól



3. a, b, c ábra

Merkel sejtes carcinoma kután áttétének szövettani vizsgálata. *a:* hematoxilin-eozin: basofil festődésű, kis, kereksejtes tumor, *b:* Perinukleáris, pontszerű CK20 pozitivitás, *c:* MCPyV ellenes antitest (CM2B4) pozitivitás

való elkülönítésben segít az S100, HMB45, MelanA és az LCA (leukocyte common antigen) markerek hiánya (22).

Staging és prognózis

A daganatos betegség stádiumának meghatározása az AJCC (American Joint Committee on Cancer) 8-as verziója alapján történik, melyet közel tízezer MCC-ben szenvedő beteg retrospektív vizsgálatára támaszkodva alkottak meg (23, 24). Az I-es stádiumba a 2 cm-es vagy kisebb tumorok (T1N0M0), a II-es stádiumba a 2 cm feletti (T2-3N0M0), valamint a környező izmokat, faszciát, porcot vagy csontot destruáló tumorok (T4N0M0) tartoznak. A regionális nyirokcsomó és/vagy in tranzit áttéttel rendelkező betegek képezik a III-as, míg távoli áttétes esetek a IV-es stádiumot.

Az MCC prognózisa a melanománál rosszabb, az 5-éves túlélés 40-60% között van, a túlélés medián értéke 8-12 hónap (23, 24). Az irodalmi adatok szerint a daganatos betegség diagnosztizálásának időpontjában a betegek 65%-ánál bőrre lokalizált a betegség, 26%-ban van nyirokcsomó érintettség, míg 8%-nál távoli metasztázis. Az 5-éves túlélés a betegcsoportoknak megfelelően 51, 35 és 14% (23, 24). Saját vizsgálatunkban az irodalmi adatokhoz képest előrehaladottabb stádiumban diagnosztizáltuk az MCC-t. Az esetek mindössze 44%-ában volt bőrre lokalizált a betegség, míg 40%-ban nodális érintettség és 16%-ban távoli áttét igazolódott a felfedezés időpontjában.

Az első két év során kb. 30%-ban számíthatunk lokális recidíva és távoli áttét kialakulására, míg regionális nyirokcsomó áttét a betegek 50%-ában jelentkezik (23, 24).

Az új európai irányelv szerint magas rizikót jelez, ha legalább egy tényező fennáll a következők közül: a tumor 2 cm-es vagy nagyobb méretű, fej-nyaki lokalizáció, krónikus immunszuppresszió, limfovaszkuláris invázió, regionális nyirokcsomó áttét (15).

A Merkel sejtes karcinóma kezelési lehetőségei

Az MCC kezelésének meghatározását multidiszciplináris onkoteam végzi. A sebészi kezelés mellett sugárterápia és szisztémás kezelés állnak rendelkezésre (15).

Sebészi kezelés

A primer tumor elsődleges ellátása a sebészi eltávolítás 1-2 cm-es biztonsági zónával és az őrszem nyirokcsomó biopszia (standard of care) (15). A tumor magas recidíva arányát tekintve a sebészi beavatkozás döntő jelentőséggel bír. A Mohs-féle mikroszkóposan kontrollált sebészet a fej-nyak régióban elhelyezkedő tumorok esetében alkalmazható eljárás.

Szentinel pozitivitás (mikrometasztázis) esetén komplettáló blokkdisszekció végzése az új európai irányelv alapján rutinszerűen nem javasolt, mert a teljes túlélést nem befolyásolja. Adjuváns sugárkezelés javasolt elsősorban, és csak bizonyos esetekben javasolják a blokkdisszekció mérlegelését (15).

Tapintható és/vagy vizsgálatokkal igazolt nyirokcsomó áttét (makrometasztázis) esetében javasolt a regionális blokkdisszekció, adjuváns sugárterápiával kiegészítve (15).

Adjuváns kezelés

Az MCC esetében a teljes tumorrezekción átesett betegeknél magas rizikójú betegség esetén adjuváns sugárterápia indítása javasolt, a műtéttől számított maximum nyolc héten belül a primer tumor és a drenáló nyirokrégió területére (50/60-55/66 Gy) (15). Az MCC sugárterápiára nagyon érzékeny tumor, ezért ez a modalitás kiemelt jelentőségű a betegség kezelése során, szinte minden stádiumban. Kevés randomizált, kontrollált vizsgálat van azonban, amely részben a tumor ritka előfordulásával magyarázható. Retrospektív adatelemzések (eset sorozatok, regiszterek) alapján a sebészi kezelést követően a primer tumorágyra adott adjuváns sugárterápia javítja a lokális és regionális relapszus-mentes túlélést, a távoli áttét-mentes túlélést és a teljes túlélést, önmagában a sebészi kezeléshez viszonyítva (15). Pozitív őrszem nyirokcsomó biopsziát követően adott adjuváns sugárterápia javítja a regionális relapszus-mentes túlélést, a teljes túlélésre való hatása nem egyértelmű (15). Makroszkópos nyirokcsomó áttét esetén posztoperatív adjuváns sugárterápia javasolt a regionális blokkdisszekciót követően, jelentősége fokozott több nyirokcsomó érintettsége és extrakapszuláris terjedés esetén (15).

Adjuváns kemoterápiát az új európai irányelv nem javasol, míg adjuváns immunterápiával klinikai vizsgálatok vannak folyamatban (15).

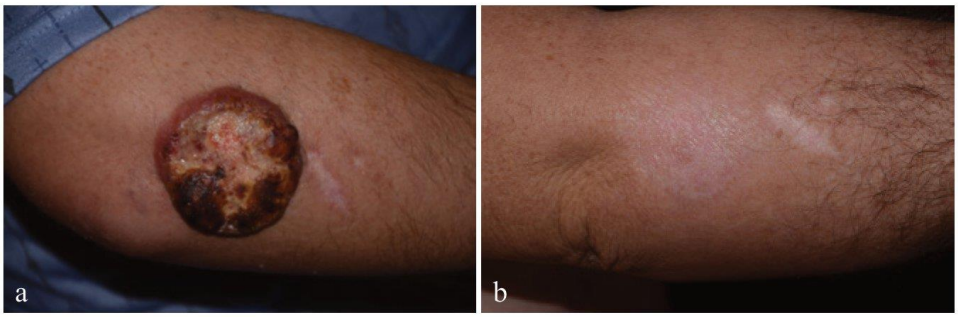
A disszeminált betegség szisztémás kezelése

Olyan esetekben, amikor a sebészi kezelés nagy megterheléssel jár, a primer tumor és a nyirokrégió palliatív sugárkezelése végezhető. A betegség azon formáiban, amikor sebészi és/vagy sugárterápia nem állnak rendelkezésre, szisztémás kezelés javasolt.

Korábban, az immunterápiás éra előtt platina, etopozid, taxán alapú kombinált kemoterápiát alkalmaztunk, melyet az általában idős betegek nehezen toleráltak. A terápiás válaszadási arány első vonalban adott kemoterápia esetén 53-61%, másodvonalban 23-45% volt, a válasz azonban nem volt tartós (elsővonal mPFS: 3,1 hó, mOS: 9,5 hó, másodvonal mPFS: 2-3 hó) (15). Jelenleg a kemoterápia másodvonalban javasolt, ha a tumoros betegség immunterápiára rezisztens vagy ha valamilyen okból kifolyólag immunterápia nem adható a betegnek.

A melanomához hasonlóan az MCC kezelésében is elsővonalbeli szisztémás terápia az immunellenőrzőpontgátló PD1/PDL1-gátló immunterápia (15). Nghiem *és mtsai.* fázis 2-es vizsgálatában elsővonalbeli kezelésként PD1-gátló pembrolizumabot alkalmaztak 25 előrehaladott MCC-s beteg kezelésében (25, 26). A vizsgálatban az objektív válaszadási arány (ORR) 56% volt, komplett remissziót 24%-ban, részleges remissziót 32%-ban tapasztaltak. A terápiás válasz időtartama (DOR) a válaszadó betegek 96%-ánál elérte a 6 hónapot, a betegek 56%-ánál legalább 12 hónap volt (25, 26). A kezelés hatékonysága a vírus pozitív és negatív tumoroknál hasonló volt.

Kaufmann *és mtsai.* egykarú, multicentrikus, fázis II-es klinikai vizsgálatában a betegek (n=88) kemoterápiát követően másodvonalban avelumab (PDL1-gátló) adásá-



4. a, b ábra
Komplett remisszió avelumab kezeléssel felső végtagi, lokoregionálisan előrehaladott Merkel sejtes carcinomában
a: Exulcerált tumor az immunterápia előtt, *b:* Komplett remisszió, a tumor helyén hipopigmentált folt

ban részesültek (27, 28). Az objektív tumorválaszt mutató betegek aránya 32% volt, 8 betegnél komplett, 20 betegnél parciális remisszió igazolódott. A terápiás válasz időtartama a betegek 86%-ánál legalább 6 hónapig, a betegek közel felénél (45%) egy évig tartott. A vizsgálatban a tumor virális státusza (MCPyV+ vagy MCPyV-) az immunterápia hatékonyságát nem befolyásolta. A vizsgálatban a túlélés medián értéke 12,6 hónap (95% CI, 7,5-17,1 hónap), a 4- és 5-éves túlélés 30% (95% CI, 20-40%) és 26% (95% CI, 17-36%) volt (27, 28). Az avelumab kezelést ugyanebben a vizsgálatban 116 beteg kapta első vonalban (29). A követési idő 21,2 hónapja (14,9-36,6) során a teljes túlélés median értéke 20 hónap (12,4-NR), a progresszió mentes túlélés 4,1 (1,4-6,1) hónap volt. A betegek 60%-a (95%CI, 50-68%) volt életben az 1 éves utánkövetéskor (29). A vizsgálat további analízisei folyamatban vannak.

Hazánkban irrezekábilis és/vagy metasztatikus MCC kezelésére elsővonalban, monoterápia formájában az avelumab immunterápia van törzskönyvezve *(4. a, b ábra)* (30). MCC-ben elmondható, hogy bár eddig a legígéretesebb eredmények immunterápiákkal születtek, de a betegek felénél még ezekkel sem sikerül tartós tumorválaszt elérni. Igazán megbízható prediktív markere a kezelésnek nincsen, talán legjobb tumorválaszt azokban a tumorokban láttak, ahol magas volt a mutációs terhelés, vírus negatív volt a tumor és magas volt a CD8+ tumort infiltráló limfociták aránya.

Adjuváns immunterápiával (PD1-gátló, PDL1-gátló, CTLA4-gátló) több vizsgálat van folyamatban, teljes tumorrezekción átesett betegeknél. Igazolt nyirokcsomó áttét (III-as stádium) esetén a műtéti kezelés előtt neoadjuvánsan adott nivolumabbal 39 beteget kezeltek klinikai vizsgálatban (31). Néhány ciklus immunterápiát követően 35 beteg esett át műtéten, közel 50%-nál szövettanilag nem sikerült reziduális tumort kimutatni (patológiai tumorválasz: 47,2%) és a 20 hónapig tartó utánkövetés során relapszus egyiküknél sem jelentkezett. Ezek alapján a neoadjuváns kezelés ígéretesnek tűnik az MCC kezelésében (31).

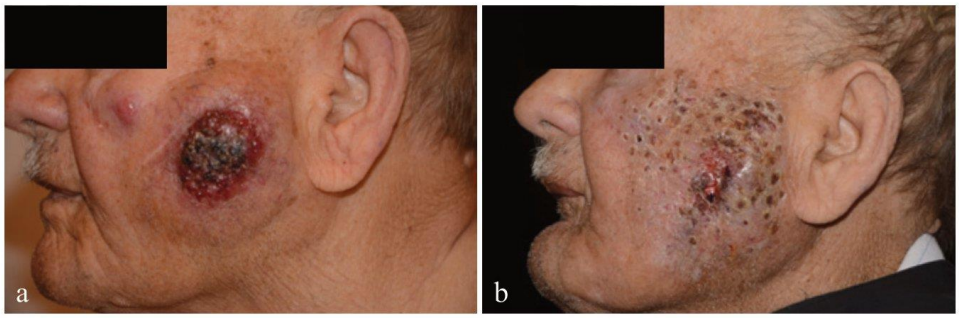
Egyéb kezelési próbálkozások

A bővülő terápiás lehetőségek ellenére a bőrön megjelenő malignus tumorok ellátása gyakran kihívást jelent az ellátó multidiszciplinális team számára. Az elektrokemoterápiát (ECT) az 1980-as évek végén vezették be (32). Az eljárás során nagy energiájú elektromos impulzus és citosztatikum (bleomicin) egyidejű alkalmazásával érhető el daganatellenes hatás. Napjainkban a bőrön és a bőr alatt elhelyezkedő primer, illetve metasztatikus daganatok kezelési armamentáriumának része (32).

Az irodalomban MCC-ben az ECT alkalmazására vonatkozóan eset-sorozatok állnak rendelkezésre. Fej-nyak régióban elhelyezkedő MCC kután és szubkután metasztázisainak palliatív ECT kezelése során a tumorok méretének csökkenéséről, egyes esetben komplett remisszióról és a betegek életminőségének javulásáról számoltak be (33, 34). MCC-ben ECT kezelést követően alkalmazott egyetlen ciklus avelumab kezelés okozott komplett remissziót. A szerzők felvetették, hogy az ECT segíthet az immunterápia számára kedvező tumor mikrokörnyezet kialakításában (35). Klinikánkon az ECT-t a progresszió késleltetése és az arcideg megkímélése céljából alkalmaztuk fej-nyak régióban lévő, gyorsan progrediáló MCC lokálisan recidívájának kezelésében *(5. a, b ábra)*.

MCC szisztémás kezelésében további kezelési próbálkozások (fázis I/II vizsgálatok) vannak metasztatikus vonalon új hatóanyagokkal (lenvatinib, T-VEC), valamint a PD1/L1-gátlók kombinációban történő alkalmazásával egyéb immunterápiás (TLR3 agonista, anti-LAG3, IL2) és kemoterápiás (paclitaxel) készítményekkel (36).

Célzott onkológiai kezelésekkel (multi-tirozináz-gátlók) is próbálkoznak az MCC kezelésében, azonban ezek eredményeire még várnunk kell, illetve igazán jól célozható támadáspontot MCC-ben egyenlőre nem sikerült találni. Az MCC, mint neuroendokrin bőrtumor, szomatosztatin 2 receptort expresszál, ezért szomatosztatin analógokkal történtek kezelési próbálkozások. A kevés rendelkezésre álló adat alapján, figyelembe véve az immunterápiát is, ezek nem tűnnek ígéretes lehetőségnek (37).



5. a, b ábra
Merkel sejtes carcinoma lokális recidívájának palliatív kezelése elektrokemoterápiával
a: A progresszió késleltetése palliatív elektrokemoterápiával. ECT kezelés előtt, *b:* ECT kezelés után 1 hónappal

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EREDETI KÖZLEMÉNY

Új eljárás a nem melanoma típusú bőrdaganatok kezelésében: „daylight” fotodinámiás terápia

Ágoston Dóra dr.[1] ▪ Ócsai Henriette dr.[1, 2] ▪ Ignácz Ferenc[3]
Viharosné Dósa-Rácz Éva[1] ▪ Rárosi Ferenc dr.[4] ▪ Oláh Judit dr.[1, 5]
Kemény Lajos dr.[1] ▪ Baltás Eszter dr.[1]

[1]Szegedi Tudományegyetem, Szent-Györgyi Albert Orvostudományi Kar, Bőrgyógyászati és Allergológiai Klinika, Szeged
[2]Békés Megyei Központi Kórház, Pándy Kálmán Tagkórház, Gyula
[3]Szegedi Tudományegyetem, Természettudományi és Informatikai Kar, Optikai és Kvantumelektronikai Tanszék, Szeged
[4]Szegedi Tudományegyetem, Szent-Györgyi Albert Orvostudományi Kar, Orvosi Fizikai és Orvosi Informatikai Intézet, Szeged
[5]Szegedi Tudományegyetem, Szent-Györgyi Albert Orvostudományi Kar, Onkoterápiás Klinika, Szeged

Bevezetés: A fotodinámiás terápia a superficialis basalsejtes carcinoma, a Bowen-kór és az aktinikus keratosis kezelésére alkalmas eljárás. A módszer lényege, hogy a kezelendő bőrfelületre felvitt fényérzékenyítő anyag metabolitjából fény hatására reaktívoxigén-gyökök szabadulnak fel, melyek szelektíven a tumorsejtek pusztulásához vezetnek. Az eljárás mesterséges fényforrással vagy a napfény alkalmazásával végezhető. Ez utóbbi a „daylight” fotodinámiás terápia, melynek hatékonysága mellett előnye, hogy nem jár fájdalommal.
Célkitűzés: Munkánk célkitűzése a napfénnyel végzett fotodinámiás terápia bevezetése volt klinikánkon aktinikus keratosis indikációjában és a kezelési protokoll optimalizálása a helyi éghajlati viszonyokhoz.
Módszer: Klinikai vizsgálatunk három részből állt. A kezelési protokollok között különbség a fényérzékenyítő anyag inkubációs idejében és a napfényen történő kezelés időtartamában volt.
Eredmények: A nemzetközi protokoll alapján végzett vizsgálatban az aktinikus keratosisok 73%-ában komplett, 27%-ában részleges remissziót értünk el. A szabadban eltöltött idő arányát csökkentve a lasiók kétharmadánál teljes, egyharmadánál részleges remissziót értünk el. 100 J/cm² feletti kezelési dózis esetén súlyos erythema megjelenését észleltük a kezelést követő 24 órában. Ennek elkerülésére dozimetria segítségével határoztuk meg a szabadban eltöltött kezelési időt. A betegek a kezelést jól tolerálták, a lasiók 15%-ában részleges, 85%-ában teljes remissziót értünk el.
Megbeszélés: A módosított nemzetközi protokoll alapján végzett „daylight” fotodinámiás terápia hatékony és jól tolerálható kezelési eljárás az aktinikus keratosis indikációjában.
Következtetés: A napfénnyel végzett fotodinámiás kezelést sikerrel adaptáltuk és alkalmazzuk klinikánkon a mindennapi gyakorlatban aktinikus keratosisok kezelésében.
Orv Hetil. 2022; 163(36): 1422–1429.

Kulcsszavak: napfény, fotodinámiás terápia, aktinikus keratosis

Innovation in the treatment of non-melanoma skin cancer: daylight photodynamic therapy

Introduction: Photodynamic therapy is indicated for the treatment of superficial basal cell carcinoma, Bowen's disease and actinic keratosis. Reactive oxygen radicals are released from the metabolite of the topically applied photosensitizer that is excited by light, which selectively leads to the destruction of tumor cells. The procedure can be performed with an artificial light source or with the use of sunlight. The latter is called daylight photodynamic therapy, which is an effective and painless procedure.
Objective: Our aim was to introduce daylight photodynamic therapy in actinic keratoses at our department and to optimize the treatment protocol for the local climatic conditions.
Method: Three clinical trials were performed. The difference between the treatment protocols was between the incubation time of the photosensitizer on the skin and in the time patients spent under the sunlight.

Results: When using the international treatment protocol, 73% of the actinic keratoses showed complete, while 27% partial remission. By reducing the proportion of time patients spent outdoor, complete remission was achieved in two-thirds and partial remission in one-third of the lesions. At doses above 100 J/cm², severe erythema was observed 24 hours after the treatment. To avoid this, we calculated the time to be spent outdoor by dosimetry. Partial remission was achieved in 15%, complete remission in 85% of the actinic keratoses with good tolerability.
Discussion: The stepwise modification of the treatment protocol resulted in an effective and well-tolerated treatment in actinic keratoses under the local climatic conditions.
Conclusion: The method has been successfully adapted in our clinic and is used in daily practice to treat actinic keratoses.

Keywords: daylight, photodynamic therapy, actinic keratosis

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Rövidítések

5-FU = 5-fluorouracil; ALA = aminolevulinsav; CR = (complete remission) komplett remisszió; DALA = delta-aminolevulinsav; d-PDT = (daylight photodynamic therapy) „daylight" fotodinámiás terápia; EDF = Európai Dermatológiai Fórum; HPV = humán papillomavírus; k-PDT = (conventional photodynamic therapy) konvencionális fotodinámiás terápia; PDT = (photodynamic therapy) fotodinámiás terápia; PpIX = protoporfirin IX; PR = parciális remisszió; UV = (ultraviolet) ultraibolya; VAS = vizuális analóg skála

A keratinocytaeredetű, nem melanoma típusú bőrtumorokon a basalsejtes és a cutan laphámsejtes carcinomát értjük, melyek a leggyakrabban előforduló humán daganatok. Incidenciájuk évről évre emelkedő tendenciát mutat világszerte [1]. Kezelésük az esetek nagy részében egy helyi érzéstelenítésben elvégzett sebészi kimetszéssel történik. A ritkán előforduló, lokálisan előrehaladott és metasztatizáló formák esetében a terápia meghatározása multidiszciplináris team feladata. A sebészi és sugárterápiás módszerek mellett szisztémás kezelések jönnek szóba, melyek közül kiemelhetők az elmúlt évtizedben megjelent, molekulárisan célzott és immunonkológiai kezelések. A hámeredetű bőrtumorok elsősorban korai, illetve felszínesebb formáiban (superficialis basalsejtes carcinoma, aktinikus keratosis) számos lokális kezelési alternatíva közül választhatunk [2]. Ezek közé tartozik a krioterápia, bizonyos lokális gyógyszeres kezelések és a fotodinámiás terápia (PDT).

Az aktinikus keratosis az Európai Dermatológiai Fórum (EDF) szerint *in situ* bőrlaphámráknak tekinthető [3]. Az aktinikus keratosis prevalenciája igen magas, Ausztráliában és a 40 év feletti kaukázusi lakosság körében 40–60% [1]. Irodalmi adatok alapján aktinikus keratosis talaján alakul ki az invazív bőrlaphámrákok 60–80%-a, ezért kezelésük kiemelt jelentőségű [4, 5].

Az evidencia erősségét figyelembe véve az aktinikus keratosisok kezelési armamentáriumába a krioterápia (magas), az 5-fluorouracil (5-FU)-krém (magas), az imikimodkrém (magas), a PDT (közepes), a CO_2-lézeres ablatio (közepes) és a diklofenákgél (közepes) tartozik [6, 7]. Sebészi eltávolítás csak szelektált esetekben javasolt. Egy 2021-ben megjelent szisztematikus vizsgálat alapján a legerősebb ajánlást az aktinikus keratosis kezelésében a krioterápia, az 5-FU-krém és az imikimiodkrém esetében fogalmazták meg [2]. Nincs azonban kellő bizonyíték a kezelések hatékonyságára immunszupprimált betegeknél, illetve arra, hogy alkalmasak-e a kemoprevencióra. A „field cancerisation" jelensége miatt gyakran nemcsak az egyes lasiók kezelésére, hanem kiterjedtebb bőrterületre irányuló (ún. field-directed) terápiára is szükség van, melyre a PDT, a lokális imikimodkrém és a krioterápia alkalmas [2].

A fényérzékenyítő anyaggal történő kezelés története az ókorig nyúlik vissza, és folyamatos fejlődése napjainkban is tart [8]. A PDT modern korszakát 1978-tól számítjuk, amikor Doughety és munkatársai elvégezték az első szisztematikus humánvizsgálatokat, melyek során PDT-vel cutan és subcutan bőrtumorokat kezeltek [8]. Napjainkban a PDT-kezelés a szakmai irányelvekben befogadott terápia az aktinikus keratosis, a superficialis basalsejtes carcinoma és a Bowen-kór esetén. Indikáción kívül alkalmazzák acne vulgaris, vírusos szemölcsök, condyloma, scleroderma és cutan leishmaniasis kórképekben, valamint használják bőrfiatalításra is.

A PDT bőrgyógyászati alkalmazása során a kezelendő bőrfelületre aminolevulinsav (ALA)-alapú fényérzékenyítő krémet viszünk fel. Meghatározott inkubációs idő után a fényérzékenyítőből képződött metabolitot, a protoporfirin IX-et (PpIX) megfelelő hullámhosszúságú fénnyel gerjesztjük. A PpIX alapállapotba való visszatérése közben reaktívoxigén-gyökök keletkeznek, ami sejtpusztulást (nekrózis vagy apoptózis) idéz elő [9]. A fényérzékenyítő anyag a gyorsan osztódó sejtekben és az újonnan képződött erekben nagyobb mértékben és gyorsabban akkumulálódik, mint a környező ép szöve-

tekben, így az eljárás szelektíven képes roncsolni a kezelt területen lévő tumorsejteket [10].

A PDT számos innováción ment keresztül az elmúlt évtizedben [8]. Az első fényérzékenyítő készítményeket intravénásan kellett adni, és hatásuk akár 2 hónapig tartott. Az ezt követő évtizedekben fejlesztették ki a napjainkban is használatos, ALA-alapú fényérzékenyítőket, melyeket lokálisan kell alkalmazni, hatékonyabbak és kevésbé toxikusak. További kutatások a készítmények stabilitásának javítását, a bőr felső rétegébe történő könnyebb és mélyebb penetrációját, valamint a kényelmesebb alkalmazhatóságot (transdermalis tapasz) célozták meg [8]. Napjainkban több 5-ALA- vagy az észter származékát tartalmazó metil-5-ALA-tartalmú lokális készítménynek van indikációja a dermatoonkológiában. A két hatóanyag közötti legfontosabb különbség, hogy az utóbbival (metil-5-ALA) végzett PDT jóval kedvezőbb a fájdalom tekintetében. Azokban az országokban, így Magyarországon is, ahol nincs forgalomban lokális fényérzékenyítő gyári készítmény, magisztrális receptúrában állítják elő.

Előrelépések történtek a PDT során alkalmazott fény tekintetében is [8]. A konvencionális PDT (k-PDT) mellett megjelent a természetes napfénnyel végzett „daylight" PDT (d-PDT). A k-PDT során a legtöbbször vörös (635 nm) vagy kék (410 nm) fényt sugárzó mesterséges fényforrást használunk a fényérzékenyítő anyag gerjesztéséhez. A természetes napfénnyel (látható fény: 380–700 nm) történő d-PDT-t először Koppenhágában alkalmazták 2008-ban *Wiegell és mtsai* [11]. Irodalmi adatok igazolják, hogy a d-PDT és a k-PDT hatékonysága a superficialis basalsejtes carcinoma és az aktinikus keratosis kezelésében hasonló, a d-PDT azonban jobban tolerálható, és lényegesen kevesebb fájdalommal jár [8, 12]. A k-PDT során a fényérzékenyítő anyagot 3 órán keresztül okklúzióban inkubáljuk a kezelt területen. A mesterséges fényforrással történő besugárzás akkor történik, amikor a PpIX akkumulációja eléri a maximumát. A nemzetközi protokoll a d-PDT során 30 perces inkubációs időt javasol okklúzió nélkül. A PpIX akkumulációja a napfénnyel történő expozíció kezdetekor nem éri el a maximumát, és fokozatosan folytatódik. Az inkrementális PpIX-aktiváció lehet az egyik magyarázata, hogy a d-PDT kevésbé fájdalmas a k-PDT-hez képest [13]. További előny, hogy a d-PDT-vel nagyobb bőrfelület kezelése lehetséges [8].

A d-PDT-t befolyásolja az időjárás. A csapadékos és hideg idő, az erős szél akadályozza a kezelés kivitelezését, a kezelés során a magas ultraibolya (UV-) sugárzás nehezen tolerálható, és fokozza a nem kívánt hatások (például bőrpír) kialakulásának valószínűségét [14]. Nemzetközi konszenzus a d-PDT-vel kapcsolatosan 2012-ben született [15]. Azóta számos ország vezette be a módszert, adaptálva az adott térség földrajzi és éghajlati viszonyaira [16–20].

Módszerek

Munkánk célkitűzése volt a természetes napfénnyel végzett PDT bevezetése a Bőrgyógyászati és Allergológiai Klinikán az aktinikus keratosis indikációjában. A kezelés hatékonyságát és nem kívánt hatásait vizsgáltuk, különös tekintettel a fájdalomra. Célunk volt továbbá egy olyan kezelési protokoll kidolgozása, amely optimális a helyi éghajlati viszonyok tekintetében is.

A vizsgálatot a Szegedi Tudományegyetem Bőrgyógyászati és Allergológiai Klinikáján végeztük a Regionális és Intézményi Etikai Bizottság jóváhagyásával (protokollszám: PDT-DLIGHT). A vizsgálatba történő legfontosabb bevonási kritériumok a 18 év feletti életkor, a fej-nyak tájra lokalizálódó, 6 mm-t meghaladó méretű aktinikus keratosis voltak. Klinikai vizsgálataink során elsősorban olyan betegeket választottunk be, akiknél egyetlen, d-PDT-vel kezelendő aktinikus keratosis volt. Multiplex aktinikus keratosis esetén „target" laesiót jelöltünk ki, melyet a klinikai vizsgálat során a hatékonyság és a mellékhatások tekintetében követtünk.

Az aktinikus keratosis diagnózisát klinikai és dermatoszkópos kép alapján állítottuk fel. Szövettani verifikáció csak szelektált esetekben történt. Kizárási kritérium volt a vizsgálatot megelőző 6 hétben a kezelendő területen alkalmazott egyéb beavatkozás (krioterápia, helyi gyógyszeres kezelés, sebészi vagy lézerkezelés, sugárterápia), valamint a fényérzékenyítő anyaggal szembeni túlérzékenység. A betegek részletes felvilágosítást követően, a beleegyező nyilatkozat aláírásával erősítették meg a vizsgálatban való részvételi szándékukat.

A természetes napfénnyel történő fotodinámiás kezelést négy lépésben végeztük. A kezelendő bőrterületet a nemzetközi protokoll alapján készítettük elő [21, 22]. Az aktinikus keratosisról a hyperkeratosist Volkmann-kanállal eltávolítottuk. Magas faktorszámú fényvédő krémet vittünk fel a kezelendő, illetve a környező bőrterületre. Fényérzékenyítő anyagként 10%-os delta-aminolevulinsavat (DALA) tartalmazó magisztrális krémet használtunk, melyet okklúzió nélkül alkalmaztunk a kezelendő területen és 5 mm-es környezetében.

A vizsgálatok során a következő képletet használtuk a kezelési dózis, a fényerősség és a kezelési idő tekintetében: dózis (J/cm²) = [fényerősség (mW/cm²) × kezelési idő (min) × 0,6] / 10. Vizsgálataink során a fényerősség mérésére a Vector H410 dozimétert (Scientech Inc., Boulder, CO, USA) alkalmaztuk.

A kezelés hatékonyságát (klinikai válasz) a klinikai és a dermatoszkópos kép segítségével értékeltük 6 hét elteltével. Komplett remissziónak (CR) tekintettük, ha a kezelt területen teljes regressziót értünk el, míg parciális remissziónak (PR), ha a lasio legalább egyharmad része regrediált. Stabil betegség fennállásáról akkor beszéltünk, ha a lasio kevesebb mint egyharmad része regrediált, míg progressziónak tekintettük, ha a kezelést követően 6 héttel az eredeti lasio növekedését láttuk.

Vizsgálataink során a klinikai választ befolyásoló egyéni tényezőket nem vizsgáltuk, és a kezelési dózisokkal való összefüggést statisztikailag nem elemeztük.

A nem kívánt hatásokat súlyosság szerint (0–4 pont: 0 = nincs, 1 = enyhe, 2 = közepes, 3 = súlyos, 4 = nagyon súlyos) a kezelés után 6 hét elteltével, illetve panasz jelentkezése esetén értékeltük. A kezelés során fellépő fájdalmat vizuális analóg skálán (VAS, 0–10 pont: 0 = fájdalommentesség, 10 = elviselhetetlen fájdalom) mértük. A kezelést esős idő, illetve 10 °C alatti és 35 °C feletti külső hőmérséklet esetén elhalasztottuk.

Összesen három klinikai vizsgálatot végeztünk d-PDT-vel (PDT-DLIGHT-001, -002, -003). A bevonási és a kizárási kritériumok, valamint a kezelés eredményeinek és nem kívánt hatásainak értékelése hasonlóan történt mindhárom vizsgálatban. A kezelési protokoll a négy lépésben megegyezett. Különbség az egyes vizsgálatok között a fényérzékenyítő anyag inkubációs idejében és a természetes napfényen történő kezelés időtartamában volt *(1. táblázat)*. A nemzetközi protokollban javasoltakhoz képest azért változtattunk a paramétereken, hogy a módszer a hazai éghajlati viszonyok között jobban tolerálható legyen betegeink számára.

A d-PDT-vizsgálatok, illetve a korábban klinikánkon k-PDT-vel végzett vizsgálat eredményeinek összehasonlítása és statisztikai elemzése az IBM SPSS 26 szoftverrel (IBM Corporation, Armonk, NY, USA) történt. Az adatok egymintás Shapiro–Wilk-próba alapján nem bizonyultak normális eloszlásúnak. A PDT hatékonyságának elemzéséhez khi-négyzet-próbát, míg a fájdalomértékek összehasonlításához a Kruskal–Wallis-próbát használtuk. A páronkénti összehasonlításokat a Dunn-féle post-hoc teszttel végeztük el. A CR-ek arányainak összehasonlítása a szignifikáns khi-négyzet-próbát követően Benjamini–Hochberg-korrekcióval történt. A szokásos 5%-os szignifikanciaszintet alkalmaztuk.

Eredmények

A PDT-DLIGHT-001-es vizsgálat során a nemzetközi protokoll alapján a fényérzékenyítő anyagot a kezelendő bőrterületen okklúzió nélkül inkubáltuk 30 percig. Ezt követően betegeink 90 percig a szabadban napfénynek voltak kitéve [23]. Összesen 63 beteg (33 férfi, 30 nő) grade I-es aktinikus keratosisát kezeltük a fej-nyak régióban (arc, fejtető, homlok, orr). A betegek átlagéletkora 75,37 év (49–92 év) volt. 1 órával a kezelést megelőzően a kültéren mért átlagos hőmérséklet 25,63 °C (12–32 °C) volt. Felhős (1–13 °C), részben napos (10–25 °C) és napos (17–31 °C) időjárási feltételek mellett végeztük a terápiát.

Az aktinikus keratosisok 73%-a (n = 46) CR-t, 27%-a (n = 17) PR-t mutatott. A kezelés során jelentkező fájdalmat a betegek a VAS-skálán 0,3 (0–5) pontra értékelték *(1. táblázat)*. A PDT-DLIGHT-001-es vizsgálat során megfigyeltük, hogy a 90 perc időtartamú kültéri tartózkodás napos és részben napos idő esetén megterhelést jelentett idős betegeink számára.

1. táblázat | A „daylight" PDT-vel (PDT-DLIGHT-1, -2, -3) és a konvencionális PDT-vel [27] *(Gaál és mtsai)* végzett vizsgálatokban részt vevő betegek, a kezelési paraméterek és az eredmények

Klinikai vizsgálat		„Daylight" PDT			Konvencionális PDT[28]
		001	002	003	
Betegek	A betegek száma (n)	63	30	73	22
	Nem (férfi : nő) (n)	33 : 30	16 : 14	47 : 26	11 : 11
	Átlagéletkor (év)	75,37 (49–92)	77,90 (41–97)	74,64 (51–92)	75,86 (62–92)
Kezelési paraméter	Fényérzékenyítő anyag	10%-os DALA			20%-os DALA
	Inkubációs idő beltéren (perc)	30	120	120	240
	Kezelési idő a szabadban (perc)	90	30	10,42 (2–60)	12 perc/mező
	Mért fényerősség (mW/cm²)	NA	48,63 (7–71)	46,67 (2–92)	NA
	Dózis (J/cm²)	NA	87,5 (12,6–127,8)	19,47 (7,2–54)	37
Klinikai válasz	Parciális remisszió, aktinikus keratosis, % (n)	26,98 (17)	36,66 (11)	15,07 (11)	31,82 (7)
	Komplett remisszió, aktinikus keratosis, % (n)	73,01 (46)	63,33 (19)	84,93 (62)	59,09 (13)
	Stabil betegség, aktinikus keratosis % (n)	0	0	0	9,09 (2)
	Progresszió, aktinikus keratosis, % (n)	0	0	0	0
Fájdalom	Fájdalom (VAS-skála: 0–10)	0,3 (0–5)	2,13 (0–10)	0	6,94 (3–10)
	Fájdalomcsillapítás, a betegek száma (n)	0	0	0	21

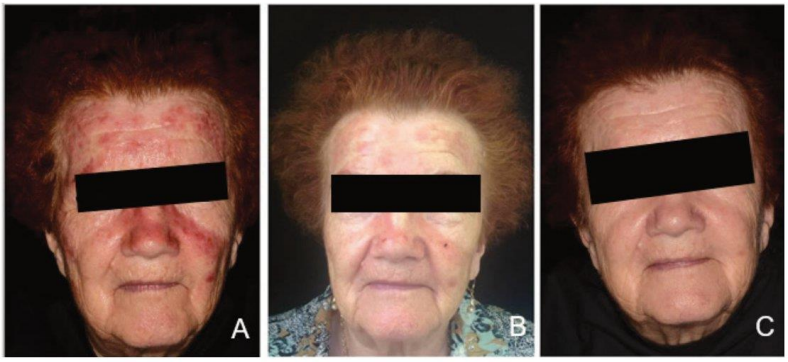
DALA = delta-aminolevulinsav; NA = nincs adat; PDT = fotodinámiás terápia; VAS = vizuális analóg skála

Ezt figyelembe véve további vizsgálatunkban (PDT-DLIGHT-002) a fényérzékenyítő anyag beltéren történő inkubációjának időtartamát növeltük, míg a szabadban töltött kezelési időt lerövidítettük. A fényérzékenyítő krém felvitelét követően 30 perc helyett 120 percet vártunk, hogy a PpIX-akkumuláció megközelíthesse a maximumát. A betegek az inkubációs idő alatt (délelőtt 9 és 11 óra között) a klinika épületén belül tartózkodtak, majd 30 percig természetes napfénynek voltak kitéve klinikánk kertjében. A kezelésekre felhős (18–29 °C), részben napos (19 °C) és napos (25–35 °C) időjárási feltételek mellett került sor.

A PDT-DLIGHT-002-es vizsgálatba 30 beteget (16 férfi, 14 nő) vontunk be, átlagéletkoruk 77,9 év (41–97 év) volt. A kezeléseket megelőzően a dozimeterrel (Vector H410) mért fényerősség átlagos értéke 48,63 mW/cm² (7–71 mW/cm²) volt. A betegek 30 percet töltöttek a szabadban, a kezelési dózis értéke átlagosan 87,5 J/cm² (12,6–127,8 J/cm²) volt *(1. táblázat)*. A kezelés hatékonyságát 6 hét elteltével értékelve az aktinikus keratosisok 63,33%-ban (n = 19) CR-t, 36,66%-ban (n = 11) PR-t mutattak. A betegek a terápiát jól tolerálták, a kezelés során jelentkező fájdalom átlagos értéke a VAS-skálán 2,13 pont (0–10) volt. A fájdalom enyhe volt, spontán megszűnt, és a kezelést egyetlen esetben sem kellett megszakítani. A betegek 33%-a (n = 10) súlyos erythemát tapasztalt a kezelést követő 24 órában, mely miatt rendelésünkön jelentkeztek. Lokális bőrnyugtató kezelés alkalmazása mellett pár nap alatt az erythema enyhült, majd teljesen megszűnt *(1. ábra)*. Azt találtuk, hogy ezen betegek kezelése napos időjárási feltételek mellett történt, magasabb dózisokkal (100 J/cm² felett). Vizsgálatunkban a 15 J/cm² alatti és feletti dózissal kezelt lasiók a klinikai válasz (CR és PR) tekintetében nem mutattak különbséget.

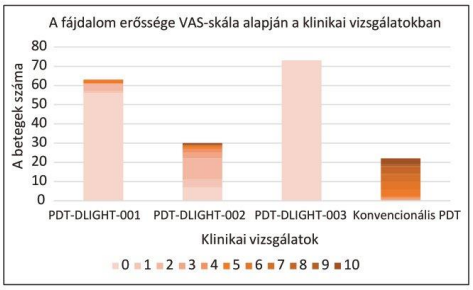
A PDT-DLIGHT-003-as klinikai vizsgálatban a fényerősség mérésére dozimetriát alkalmaztunk. Figyelembe véve a 100 mJ/cm² feletti dózisoknál tapasztalt súlyos erythemát, további vizsgálatunkban alacsonyabb kezelési dózisokat alkalmaztunk. A vizsgálatba 73 beteget (47 férfi, 26 nő) vontunk be, átlagéletkoruk 74,64 év (51–92 év) volt. Az átlagos fényerősség 46,67 mW/cm² (2–92 mW/cm²) volt. A szabadban töltött átlagos kezelési idő 10,42 perc (2–60 perc) volt. A kezelések során a betegek átlagosan 19,47 J/cm² (7,2–54 J/cm²) dózisú kezelést kaptak *(1. táblázat)*. A kezelés során fájdalom és súlyos erythema nem jelentkezett. A 6 hetes kontrollvizsgálat alkalmával a kezelt aktinikus keratosisok 85%-a (n = 62) CR, 15%-a (n = 11) PR klinikai választ mutatott.

Statisztikai elemzéssel megállapítottuk, hogy a négy csoportban mért fájdalomértékek között szignifikáns a különbség (p<0,001, Kruskal–Wallis-próba). A Dunn-féle post-hoc analízis alapján a klinikánkon korábban végzett k-PDT esetén szignifikánsan nagyobb fájdalom jelentkezett (p <0,001), mint jelenlegi d-PDT-vizsgálataink során *(2/a ábra)*. A PDT-DLIGHT-001-es vizsgálatunkban alkalmazott nemzetközi d-PDT-protokoll és a PDT-DLIGHT-003-as vizsgálatban alkalmazott módosított protokoll alapján végzett kezelések között nem volt szignifikáns különbség a hatékonyság tekintetében (p = 1). A PDT-DLIGHT-003-as protokoll alapján végzett d-PDT szignifikánsan hatékonyabbnak bizonyult a k-PDT-hez képest (p = 0,003, khi-négyzet-próba) *(2/b ábra)*. A khi-négyzet-próba szignifikáns eltérést mutatott a CR-ek arányai között a négy csoportban (p = 0,003), a legnagyobb arányban (84,9%) a PDT-DLIGHT-003-as protokoll vezetett CR-hez. A szignifikáns khi-négyzet-próbát követően a CR-ek arányaira végzett összehasonlítás alapján a PDT-DLIGHT-003-as

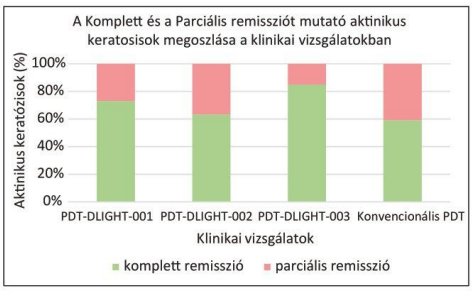


1. ábra A 79 éves nőbetegen multiplex aktinikus keratosisok „daylight" PDT-jét (dózis: 108 J/cm²) követően 24 óra múlva jelentkező súlyos erythema (A). Az erythema 5 nap lokális kezelést követően mérséklődött (B). A 6 hetes kontroll során erythema nem volt, az aktinikus keratosisok komplett remisszióját észleltük (C)

PDT = fotodinámiás terápia



a)



b)

2. ábra A „daylight" PDT-vel (PDT-DLIGHT-1, -2, -3) és a konvencionális PDT-vel [27] *(Gaál és mtsai)* végzett vizsgálatokban a fájdalom és a hatékonyság összehasonlítása. a) A kezelés során észlelt fájdalom VAS-skálán mért értékeinek összehasonlítása (a szín sötétedése a fájdalom erősödését jelzi). b) Az aktinikus keratosisok tekintetében észlelt klinikai válaszok (komplett és parciális remisszió) összehasonlítása

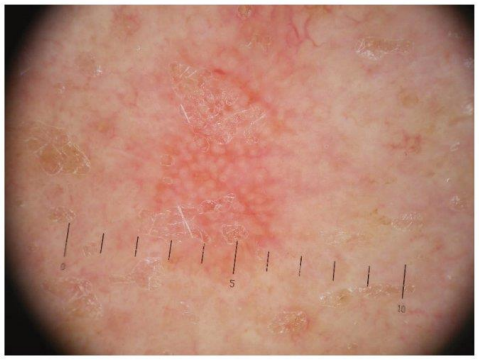
PDT = fotodinámiás terápia; VAS = vizuális analóg skála

protokoll (84,9%) és a k-PDT-protokoll (59,1%) között szignifikáns a különbség (p = 0,045) Benjamini–Hochberg-korrekcióval.

Megbeszélés

Az aktinikus keratosist 1826-ban Dubreuilh írta le először [24, 25]. Klinikailag az UV-fénynek kitett bőrfelületen, elsősorban az arcon, a fejbőrön jelenik meg, de előfordulhat a kézfejen, az alkaron és a lábszáron is. A bőrön vörösesbarna, változó mértékben keratoticus felszínű plakk formájában jelentkezik, három súlyossági fokozatát különböztetjük meg (grade I–III.). Az aktinikus keratosis sokszor multiplex előfordulású, illetve gyakori a kiterjedt, összefüggő bőrfelületet érintő, ún. „field cancerisation" jelensége. A diagnózist a klinikai és a dermatoszkópos kép *(3. ábra)* alapján állítjuk fel, szövettani verifikálást csak bizonyos esetekben végzünk [26].

A kumulatív UV-sugárzás mellett az aktinikus keratosis kialakulására hajlamosít a világos bőrtípus (Fitzpatrick I-es, II-es), az előrehaladott életkor, korábbi esetleges



3. ábra Jellegzetes dermatoszkópos kép aktinikus keratosisnál az ún. „szamócamintázat". A sárga szarudugóval kitöltött, tág follicululusok körül fehér udvar látható, melyek között az erek rózsaszínű hálózatot képeznek

arzén- vagy kátrányexpozíció, az immunszuppresszió (például szervtranszplantáltak, hematológiai betegek), a humánpapillomavírus (HPV)-infekció és bizonyos genodermatosisok (például xeroderma pigmentosum).

Aktinikus keratosisok kezelésében a PDT ígéretes kezelési eljárás. A k-PDT-hez képest a d-PDT egyszerűsített kezelés eljárás. A k-PDT esetében a fényérzékenyítő anyagot a bőrön 3 órán keresztül kell okklúzióban inkubálni, míg a d-PDT esetében 30 percig okklúzió nélkül. A másik lényeges különbség, hogy a gerjesztéshez használt fény d-PDT esetében a napfény, k-PDT esetében mesterséges fényforrás. A d-PDT előnye, hogy lényegesen csekélyebb a fájdalom a k-PDT-hez képest. A d-PDT-kezelés nemzetközi protokollja azon klinikai vizsgálatokon alapul, melyeket többségében Észak-Európában lévő centrumokban végeztek. Mivel a d-PDT szabad téren történik, és az időjárási körülmények befolyásolják a kezelés kivitelezését, hatékonyságát és elviselhetőségét, szükség van a módszer adaptálására a helyi éghajlati viszonyokhoz.

Munkánk célkitűzése a d-PDT bevezetése volt klinikánkon aktinikus keratosisok kezelésére. A módszer tekintetében a nemzetközi protokollt vettük alapul, melyet eredményeink függvényében folyamatosan változtattunk és adaptáltunk a helyi éghajlati viszonyokra. A k-PDT-vel klinikánkon 2003. december óta végzünk kezeléseket rutinszerűen, tapasztalatainkról *Gaál és mtsai* számoltak be [9, 27].

A d-PDT-vel történt vizsgálatokban betegeink átlagéletkora 75 év körül volt. A d-PDT-t a nemzetközi protokoll alapján alkalmazva az aktinikus keratosisok 73%-ában CR-t, közel 27%-ában PR-t értünk el. Idős betegeink nehezen tolerálták, hogy a természetes napfényen 90 percig kell tartózkodniuk. Protokollunkat ezt követően módosítottuk, változtattunk a klinikán és a szabadban eltöltendő időtartam arányán, míg a kezelés

teljes hossza lényegesen nem változott (120 perc *versus* 150 perc). A protokoll módosítása a kezelés hatékonyságán nem változtatott, az aktinikus keratosisok kétharmadánál CR-t, egyharmadánál PR-t értünk el. A 30 perces kezelési idővel történő d-PDT-t követően betegeink 30%-a a kezelést követő 24 órában jelentkező súlyos erythema miatt soron kívül megjelent rendelésünkön. A kezelési dózisokat elemezve megállapítottuk, hogy azon betegeinknél jelentkezett súlyos erythema, akik 100 J/cm² feletti dózisokban részesültek. Protokollunkat tovább módosítottuk, dozimetriát építettünk be, és a magas kezelési dózisok elkerülésére törekedtünk. Ezt követő vizsgálatunkban az átlagos kezelési dózis 20 J/cm² körül volt, az átlagos kezelési idő 10 perc volt. Az aktinikus keratosisok 85%-ában CR-t, 15%-ában PR-t tapasztaltunk. Megállapítottuk, hogy a módosított protokoll alapján végzett d-PDT, melynek során a szabadban töltött kezelési időt optimalizáltuk, és alacsonyabb kezelési dózisokra törekedtünk, a hatékonyságában változatlan, ugyanakkor jobban tolerálható az aktinikus keratosisban szenvedő, általában idős betegpopuláció számára. A kidolgozott protokoll alapján a d-PDT-módszert bevezettük klinikánkon a rutinszerű alkalmazásba. A d-PDT során a kezelési dózis tekintetében az alsó küszöbértékre van ajánlás, mely 8 J/cm² [19]. Az irodalmi adatok és saját vizsgálataink alapján a következő protokollt dolgoztuk ki. A kezelni kívánt bőrterületről a hyperkeratosis eltávolítása javasolt. A kezelendő és a környező bőrterületre magas faktorszámú fényvédő krém javasolt. A kezelés során a 10%-os DALA-tartalmú magisztrális krémet 120 percig inkubáljuk a bőrön okklúzió nélkül. A kezelési dózis felső küszöbértéke 100 J/cm², míg az irodalmi adatokat figyelembe véve alsó küszöbértéke 8 J/cm². Dozimetria segítségével határozzuk meg a szabadban eltöltendő kezelési időt. A d-PDT-t május és október között végezzük. Esős idő, 10 °C alatti vagy 35 °C feletti időjárás esetén a kezelést nem végezzük.

A PDT-t cutan és subcutan tumorok kezelésére 1978-ban alkalmazták először. A módszer azóta jelentős fejlődésen ment keresztül, az innováció napjainkban is folyamatosan tart. A d-PDT kivitelezését bizonyos időjárási körülmények akadályozzák, ami egyes országokban jelentősen limitálja a módszer alkalmazhatóságát [28, 29]. Ezen probléma kiküszöbölésére d-PDT-kezeléssel próbálkoztak üvegházban, illetve beltéren olyan fényforrásokat alkalmaztak, amelyekkel szimulálni lehet a napfényt [30]. A kezelési dózis meghatározásához szükséges fényerősség mérésére csuklóra rakható személyes dozimétereket (personal electronic dosimeter wristwatch, SunSaver UV dosimeter) fejlesztettek ki. A d-PDT otthoni alkalmazásának jelentősége már korábban is körvonalazódott, melyet a pandémia megerősített. A beteg által otthon elvégezhető kezelést segíti az okostelefonra letölthető applikáció (SmartPDT) [31]. Az alkalmazás műholdas alapú, közel valós idejű dozimetrián alapuló méréssel meghatározza a kezelés ideális napját és a szabadban eltöltendő időt. A kezelés körülményeit és paramétereit a beteg és a kezelőorvos is tudja követni az applikáció segítségével. Lehetőség van a kezelt bőrelváltozásról fotó feltöltésére, mely alapján teledermatológia keretén belül a bőrgyógyász meg tudja ítélni, hogy szükséges-e további kezelés.

Következtetés

A bőrlaphámrákok tekintetében kiemelkedő fontosságú az aktinikus keratosisok kezelése. A PDT olyan módszer, amely sorozatos innováción ment keresztül. Az eljárás fejlesztése napjainkban is folytatódik. Vizsgálataink során a d-PDT nemzetközi protokollját adaptáltuk. Eredményeink alapján a kezelés hatékony az aktinikus keratosis indikációjában, és betegeink számára jól tolerálható a helyi éghajlati viszonyok között.

Anyagi támogatás: A közlemény megírása, illetve a kapcsolódó kutatómunka anyagi támogatásban nem részesült.

Szerzői munkamegosztás: A klinikai vizsgálat elvégzése, „daylight" fotodinámiás terápia gyakorlati alkalmazása: Á. D., B. E. Kontroll bőrgyógyászati vizsgálat: Á. D. Kontroll onkológiai szakvizsgálat: Ó. H., B. E., O. J. Dozimetria alkalmazása: I. F. Szakmai irányelvek: K. L., B. E. Statisztikai elemzés: V. D.-R. É., R. F. A cikk végleges változatát valamennyi szerző elolvasta és jóváhagyta.

Érdekeltségek: A szerzőknek nincsenek érdekeltségeik.

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(Ágoston Dóra dr.,
Szeged, Korányi fasor 6., 6720
e-mail: agoston.dora@med.u-szeged.hu)