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Use of Targeted Osmotic Lysis for the Treatment of Cutaneous Squamous Cell Carcinoma

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Introduction

Targeted Osmotic Lysis (TOL) has been recently proposed as a novel, safe, and effective method for treating advanced carcinomas. The technique takes advantage of the finding that carcinomas greatly over-express Voltage-Gated Sodium Channels (VGSCs), a feature that confers a selective advantage for invading normal tissues and for metastasizing and a makes advanced carcinomas uniquely susceptibility to TOL. The simultaneous activation of VGSCs and pharmacological blockade of sodium, potassium-ATPase (Na+, K+-ATPase; sodium pumps) with a cardiac glycoside in highly malignant cancer cells causes intracellular hypernatremia and a sufficient increased osmotic pressure to produce lysis of cells that over-express VGSCs while leaving normal cells unaffected [1,2]. Observations made in preclinical studies, in the veterinary treatment of companion animals, and in one human subject, have indicated that TOL can reduce the size or slow the growth of advanced carcinoma, increase expected survival, and subjectively improve measures of quality of life, e.g., increased energy, increased appetite, increased social interaction, without producing significant adverse effects or damaging normal tissue. Based upon the presence of several predisposing physical and genetic traits, a history of significant sun exposure, frequent previous occurrences of skin cancer, years of research, and a review of the promising early results of TOL treatment, a 66- year-old male chose to undergo treatment of widely-distributed, cutaneous squamous cell carcinomas in the hope of reducing the frequency of having to undergo Mohs surgical resection despite the use of currently available therapeutic treatments.

Case Report

Clinical history

The patient had a history of multiple facial and torso squamous cell carcinomas over a period of 25 years with more than 15 requiring Mohs surgical resection. The first lesions were a basal cell carcinoma (BCC) of the face and a squamous cell car-

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cinoma (SCC) within his polio vaccination scar. The BCC shed its outer epidermis weekly. The SCC appeared as a 3 mm red spot. Neither lesion was painful. Biannual examinations regularly revealed either actinic keratoses (AKs), BCC or SCC lesions of the face, shoulders, chest or back. The size of cancerous lesions ranged from 2 mm to 1.2 cm. AKs were frozen and cancerous lesions were biopsied and excised with Mohs surgery.

Most recent evaluation – Four SCCs were verified by biopsy (Fig. 1, blue arrows). Several precancerous lesions were also identified (Fig. 1, green arrows). The site between the bridge of the nose and the right eye was a previous SCC treated with Mohs surgery. Three scars from other previous Mohs surgery are depicted with red arrows. There were also several erythematous patches apparent.

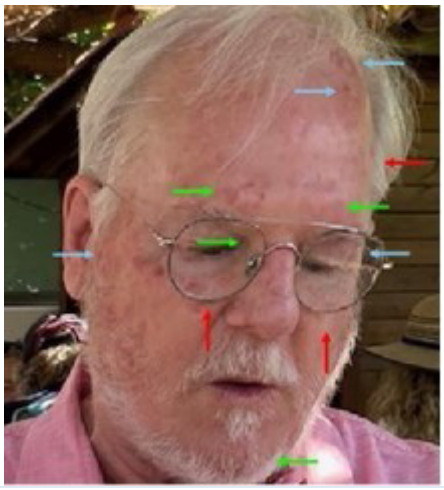

Figure 1: The photograph depicts the appearance of the skin prior to treatment with TOL. Biopsy confirmed SCCs are identified by blue arrows; lesions without biopsies by green arrows; Mohs surgery scars by red arrows. In retrospect, the erythematous patches depicted here may be angioneogenic development being established at sites of latent or undeclared lesions that have not yet reached the surface and were as yet not palpable.

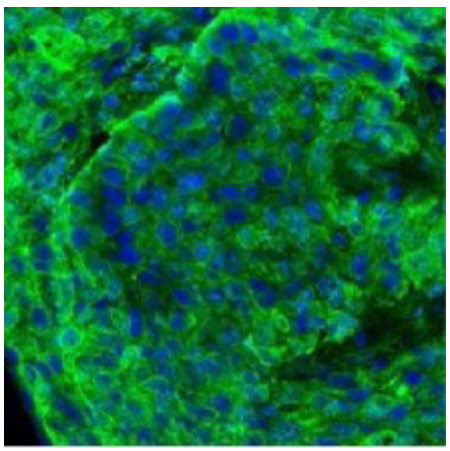

Figure 2: The photomicrograph depicts the immunohistofluorescence associated with the VGSC overexpression (green). Blue reactivity is DAPI labelling of nuclei. Calibration bar=25µm.

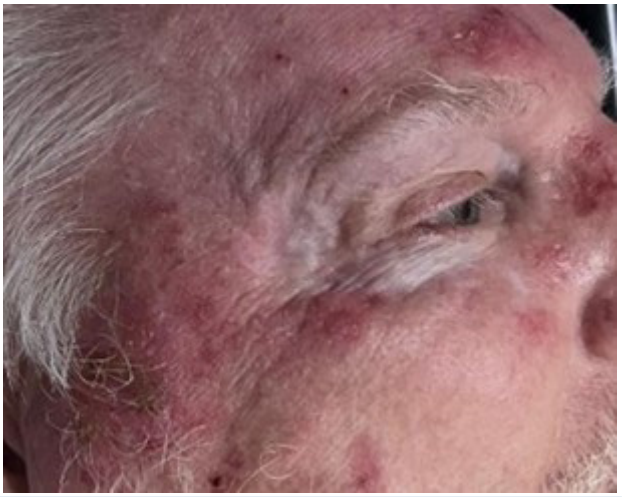

Figure 3: The photograph depicts the appearance of the skin at the first 20 min of exposure to PEF. Erythematous patches became more prominent at 10-15 min of PEF.

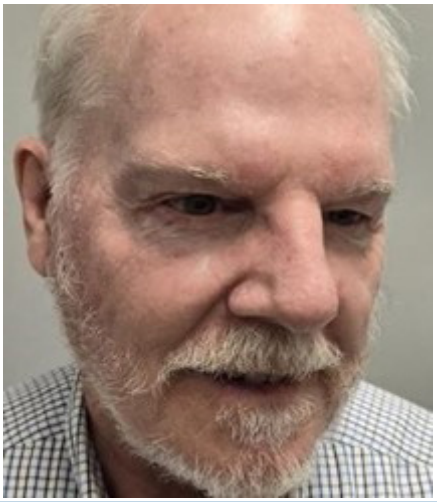

Figure 4: The photograph depicts the appearance 6 weeks after the final treatment.

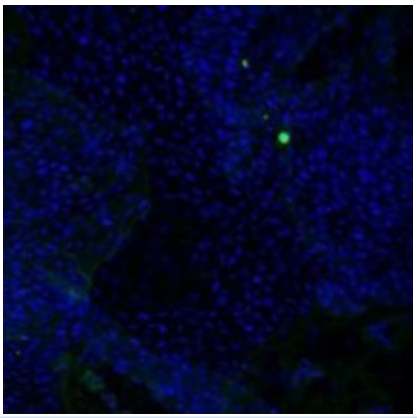

Figure 5: The photomicrograph depicts the representative level of immunohistofluroescent labeling of VGSC expression in a post-treatment tissue sample obtained 15 days after the last cycle of TOL. Only 2-3 cells in each of the 8 sections taken from the sample were found to over-express VGSCs. Calibration bar=50 µm.

Preparation, treatment and response

The patient followed the protocol approved by the GARM International Foundation Institutional Review Board for a pilot study to identify the optimum cancer-type to propose for an FDA-sanctioned "early efficacy" trial of treating advance carcinoma with TOL, to assess safety, tolerability, and survival and the effect on quality of life. Having originally proposed the application of TOL for treating cancer, being involved in the development of the technique and treatment protocol, and understanding the potential risks and benefits of TOL treatment, Informed Consent was given to undergo treatment and for obtaining a tissue biopsy to determine the level of VGSC expression in the tumor. Standard hematoxylin and eosin staining histopathological evaluation confirmed the existence of squamous cell carcinoma, and immunohistofluorescence analysis of a tumor biopsy revealed pre-treatment levels of VGSC expression relative to stromal cells to be similar to those observed in pre-clinical trials indicating that the patient's cancer sufficiently over-expressed VGSCs to anticipate a favorable response to TOL treatment (Fig. 2).

Eight days prior to undergoing the first treatment with TOL, the patient applied imiquimod daily to identified squamous cell, pre-cancerous, and actinic keratotic lesions, per routine treatment regimen provided by his dermatologist. Imiquimod induces release of the inflammatory mediators, tumor necrosis factor α (TNFα), prostaglandins, and interleukins. These mediators have been shown to increase expression of VGSCs (unpublished observation). A prescription for the cardiac glycoside, digoxin, 0.25 mg was obtained, and daily oral administration was started 5 days prior to and on each day of treatment in order to achieve and maintain a therapeutic blood level (0.50-1.50 ng/ml). The patient was exposed to a uniform pulsed electric field (PEF; 18 V/m, 10 msec positive/10 msec negative square wave, 15 msec interstimulus interval) that has been shown to effectively activate VGSCs, for 45 min on day 1 and 2 hours on day 2 in a custom-built coaxial ring device (The Phantom Laboratory, Salem, NY). [3]. Two additional treatment regimens were given, each consisting of two- hour treatments on two consecutive days preceded by five days of digoxin and imiquimod treatment.

The patient pretreated with daily oral digoxin (0.25 mg.) for five days to achieve steady-state pharmacokinetics before arriving at the clinic. After setting up the coaxial ring stimulator on site and instructing GARM staff on how to operate it, patient DP, began his first TOL treatment. He removed all metal and electronics from his person, then entered the device. Initially, the patient noted neither pain nor discomfort and did not perceive the pulsing electric field. At 15 minutes into the stimulation period, the patient began to notice a feeling of warmth and the development of patches of erythema that were accompanied with a tingling or pins and needles sensation all of which were associated with many of the previously identified lesions (Fig. 3). Shortly thereafter, serous fluid was observed to leak from visible lesions. Interestingly, the erythema and tingling were not restricted to sites on the face that were known to have cancerous lesions, but it was noticed in areas that had not

been treated with imiquimod in a remote location on the left forearm and a spot on the crown of his he**A**ad. Notably, skin color and texture continued to improve after the last treatment in that few erythematous patches remained and there was no evidence of additional scaring in areas where palpable tumors had sloughed off (Fig. 4). To date, the patient has experienced no apparent ill effects associated with the TOL treatment. A post-treatment biopsy taken 15 days after his final treatment with TOL revealed normal skin morphology and no evidence of squamous cell carcinoma (Fig. 5). Only evidence of a small basal cell carcinoma remained. Post-treatment immunohistofluroescent labeling of VGSC expression observed in each of 8 sections processed from the same tissue sample revealed that only 2-3 cells in each section were found to over-express VGSCs (Fig. 5). Because all lesions were resolved, statistics were not possible (required division by 0).

Immunohistofluorescence of VGSCs

Tissue taken at biopsy was incubated in 0.5% paraformaldehyde for 15 min., followed by 5 min in Phosphate Buffered Saline (PBS), then sectioned and mounted to coverslips. Sections were incubated for overnight in a primary panspecific antibody for VGSCs (1:800; Alomone Labs; www.alomone.com). The sections were incubated for 2 X 5 min in PBS. After removal of the primary antibody, the cells were rinsed with 0.1 mol/L phosphate buffered saline and then were incubated for 1 hour at room temperature with a 1:200 dilution of a goat-anti- rabbit secondary antibody conjugated to the Alexa Fluoro-488 fluorophore (Invitrogen, www. thermofisher.com: #A-11008). Sections incubated with the secondary antibody alone served as controls for nonspecific labeling. Sections were imaged using a HC PL APO CS2 20X/0.75 DRY objective lens on a Leica SP-2 confocal microscope (www.leica-microsystems.com). Pre- and post- treatment images were produced using identical exposure times and imaging parameters.

Microscopic analysis was performed in the xyz mode, with a 1.27 zoom and a 56.6 µm pinhole (pinhole Airy=1.00 Au). Excitation was initiated using a 488 nm (18.2%) line of an argon laser, on at 0.0000%, and photomultiplier tube set to detect an ALEXA 488 band-width, with a gain of 1241.7 and an offset of 0.13.

Discussion

With this case we report evidence that supports targeted osmotic lysis as a non-invasive option for treating cutaneous squamous cell carcinoma. TOL is based on the observation that many cancers over-express VGSCs and Na+, K+-ATPase [4-7], a feature that imparts enhanced ability to invade normal tissue and to metastasize [1,8-12]. This patient exhibited extreme up-regulation of VGSCs (Fig. 2), which was augmented by imiquimod pretreatment.

TOL offers a fundamentally different approach to the treatment of cancer than current treatment methods [13-22] by stimulating the over-expressed VGSC activity rather than destroying all cells that express the targeted feature, thus increasing the influx of sodium while simultaneously preventing the extrusion of these ions by blocking the sodium pumps [1,2]. The osmotic influx of water floods the cells beyond their capacity to comply, resulting in cell lysis [23], whereas normal cells are spared because sodium channel expression is far less than that found in most advanced carcinomas [1].

Our results here are consistent with earlier observations that TOL is well-tolerated, safe, and effective treatment that kills malignant cells without damaging normal tissue or compromising quality of life. This is clearly important when treating highly visible and potentially disfiguring cancers because TOL spares the normal cells in the surrounding supporting matrix, allowing for rapid re- epithelialization without leaving a scar.

The reported episodes of tingling were non-serious, minor intensity, short time to resolution adverse events, as they could signal some degree of peripheral nerve injury. Conversely, for many reasons, the tingling episodes may reflect normal neurological perception of cancer cell lysis. First, the tingling or sticking perception is associated with the activation of A-β or A-δ neurons, that respond to relatively low threshold stimulation rather than a burning dysesthesia associated with C-fiber stimulation and tissue damage, deafferentation pain, and liquid nitrogen destruction of cutaneous lesions. Second, the tingling sensation only occurred when the PEF was on and was perceived in limited areas associated with areas of erythema or in areas where tumors were known or likely to be present. If the tingling were due to nerve injury produced by TOL, one would expect a more generalized rather than localized distribution of the tingling, similar to the distribution of a rash produced in association with a systemic allergic drug reaction, because of the systemic, steady-state distribution of digoxin and the whole-body exposure to the PEF. Third, the tingling was perceived only when the PEF was being delivered and subsided within 10 sec. after termination of the PEF. Predictably, if the tingling sensation were associated with neurodestructive stimulation, the sensation would more likely be appreciated beyond the period of stimulation, would be appreciated at greater intensity and more widely distributed, or associated with areas of anesthesia with subsequent treatments. By contrast, the tingling sensation decreased in distribution and intensity with successive periods of stimulation and were not associated with delayed development of "numbness" or deafferentation pain.

It is unclear the degree that concurrent topical imiquimod, which induces inflammatory cytokines [24], enhanced the effectiveness of TOL, but the 8-day treatment used in this case was shorter than the 4-16-weeks required for benefit in patients with squamous or basal cell carcinomas.

Finally, we present evidence that TOL has the potential for treating a wide range of carcinomas and across an array of species [25]. By using a sodium pump blocking drug that is absorbed and distributed throughout the circulatory system and a whole-body PEF stimulation device, TOL may treat and eliminate metastases that are too small to be detected by current imaging technologies, thus reducing reappearance of carcinomas.

Author declarations

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Study approval statement

The study was conducted according to the guidelines of the GARM International Foundation Institutional Review Board, GARM #12132023 - Targeted Osmotic Lysis in Advanced Carcinoma: A Patient Pilot Study, approval date - 12/14/2023.

Consent to publish statement

The authors obtained written consent from the patient (first author) for his photographs and medical information to be published in print and online and with the understanding that this information may be publicly available. Patient consent forms were not provided to the journal but are retained by the authors.

Author contributions

Conceptualization, Dennis Paul and Harry J. Gould, III; Data curation, Dennis Paul and Harry J. Gould, III; Formal analysis, Dennis Paul and Harry J. Gould, III; Investigation, Dennis Paul and Harry J. Gould, III; Imaging, Abigail Sims; Biopsies, diagnosis, and imiquimod treatment, Leonard Gateley; Methodology, Dennis Paul and Harry J. Gould, III; Project administration, Dennis Paul; Supervision, Dennis Paul and Harry J. Gould, III; Validation, Dennis Paul and Harry J. Gould, III; Visualization, Dennis Paul, Abigail Sims, and Harry J. Gould, III; Writing – original draft, Harry J. Gould, III and Dennis Paul; Writing – review & editing, Dennis Paul and Harry J. Gould, III.

Data Availability Statement

The data presented in this study are available on request from the corresponding authors.

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