Disulfonated tetraphenyl chlorin ($TPCS_{2a}$)–induced photochemical internalisation of bleomycin in patients with solid malignancies: A first-in-man phase I dose escalation clinical trial

Ahmed A Sultan[1*], Waseem Jerjes[2*], Kristian Berg[3], Anders Høgset[4], Charles A Mosse[1], Rifat Hamoudi[2], Zaid Hamdoon[1], Celia Simeon[5], Dawn Carnell[6], Martin Forster[6,7], Colin Hopper[1,6,7]

***Both authors have equally contributed to this work**

[1] Academic Unit of Oral and Maxillofacial Surgery, UCL Eastman Dental Institute, London, UK
[2] UCL Department of Surgery, London, UK
[3] Department of Radiation Biology, Oslo University Hospital, Oslo, Norway
[4] PCI Biotech AS, Lysaker, Norway
[5] Cancer Clinical Trials Unit, University College London Hospitals, London, UK
[6] Head and Neck Unit, University College London Hospitals, London, UK
[7] UCL Cancer Institute, London, UK

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Corresponding author:
Colin Hopper
Head and Neck Unit
University College London Hospitals
London, UK
Tel: 020 3447 2159
Fax: 020 3447 2152
Email: c.hopper@ucl.ac.uk

Abstract

Introduction: Photochemical internalisation (PCI), a novel minimally invasive treatment, has shown promising preclinical results in enhancing and site-directing the effect of anti-cancer drugs by illumination. The PCI treatment involves the systemic administration of a photosensitiser followed by a chemotherapeutic agent. Illumination is then carried out with a laser of a specific wavelength, to initiate localized chemotherapy release. We report the use of a new photosensitiser $TPCS_{2a}$ (Amphinex®)-mediated photochemical internalisation (PCI) in a phase I dose-escalation trial involving patients with advanced and recurrent malignancies. The aim of the study was to assess the safety and tolerability of $TPCS_{2a}$ – mediated PCI with bleomycin and document preliminary anti-tumour activity.

Materials and methods: Patients were administered $TPCS_{2a}$ on Day 0 by slow intravenous injection, followed by a fixed dose of 15,000 IU/m^2 bleomycin, given as an infusion on Day 4. After 3 hours, the surface of the target tumour was illuminated with 652nm laser light (fixed at 60 J/cm^2). The $TPCS_{2a}$ starting dose was 0.25 mg/kg and then was escalated in successive dose cohorts of 3 patients. Adverse events were graded using Common Terminology Criteria for Adverse Events (CTCAE) v3 and in addition $TPCS_{2a}$ pharmacokinetics (PK), pain scores and skin photosensitivity testing were performed. Tumour response in target lesions was reported using RECIST 1.1 criteria.

Results: Twenty-two patients were recruited into the study and twelve patients completed the 3 months follow-up period. The administration of $TPCS_{2a}$ was found to be safe and tolerable by all patients. PCI-related adverse events were either local, due to the local inflammatory process, or systemic, mostly due to the skin photosensitising effect of $TPCS_{2a}$. Higher transient pain response localised to the treatment site was observed. No PCI-related deaths were recorded in the study.

Dose-limiting toxicities (DLTs) were observed in two patients at a $TPCS_{2a}$ dose of 1.5 mg/kg (skin photosensitivity and wound infection); thus the MTD (maximum tolerated dose) of $TPCS_{2a}$ was determined to be 1.0 mg/kg. $TPCS_{2a}$ was noted to have a rapid initial elimination followed by a slow elimination rate (half-life 15-22 days) and was still detectable in blood 90 days after administration. There were no reported photosensitivity reactions reported at 500 lux exposure (bright indoor light); whilst at 100,000 lux (direct sun light) photosensitivity was detected in at least one patient in all dose cohorts except at 0.125 mg/kg.

Dramatic tumour responses were seen, even within this heavily pre-treated patient population with advanced and recurrent malignancies. At Day 28, complete response (CR) was observed in 11 patients (58%), partial response in 2 (10%), stable disease in 2 (10%) and progressive disease in only one patient (5%). All the 0.25 mg/kg cohort patients showed complete response.

Conclusion: The $TPCS_{2a}$ -mediated PCI with bleomycin is safe and tolerable by all patients. Significant anti-tumour effects were seen with all the doses tested on several different types of tumours, warranting further clinical studies with the PCI technology. Based on our data, we have identified $TPCS_{2a}$ with the dose of 0.25 mg/kg as the recommended treatment dose for all future trials.

Introduction

Photochemical internalization (PCI) is a novel technology that facilitates the delivery of therapeutic molecules into the cytosol of cells. It was developed to enhance targeted intracellular delivery of therapeutics that are not able to penetrate cellular membranes, including proteins, nucleic acids, and various nanoparticles, as well as some small molecule chemical entities. These molecules are taken up into cells by endocytosis and accumulate in endosomes and lysosomes where they are trapped or degraded, hence, unable to exert their therapeutic potential (1). PCI aims to overcome this hurdle by utilizing highly amphiphilic photosensitisers that are trapped in the same endocytic vesicles as the therapeutics. Upon exposure to light of appropriate wavelength, reactive oxygen species are induced, rupturing the endosomes and lysosomes and thereby releasing the contents into the cytosol, allowing the therapeutics to reach their targets. By site-directed illumination, PCI can be used to target drugs preferentially to tumour sites, reducing side effects in distant normal tissues. The PCI mechanism and practical application was initially described in preclinical models by Berg et al. in 1999, highlighting potential clinical usefulness in delivering cancer therapy, gene therapy and vaccination (2).

Results from the *in vitro* evaluation showed that PCI can enhance cellular uptake of chemotherapeutic agents, with difficulty diffusing through the cellular membranes, resulting in a reverse of the multidrug resistance (MDR) process (3). *In vivo* animal PCI studies have looked at various therapeutic parameters and their outcomes. This includes tumour response, tumour selectivity and immunological response. PCI demonstrated a synergistic effect when combined with radiotherapy or after surgery. *In vivo* studies using PCI on animal models resulted in good treatment outcomes. $AlPcS_{2a}$ based gelonin PCI was found to cause significant retardation in tumour growth compared to samples treated with PDT alone (4).

Both *in vitro* and *in vivo* models have shown PCI to enhance the effect of many types of macromolecules (1,-10) and also of some small molecule anti-cancer drugs (11-13). The photosensitisers used in PCI have no serious toxic effects in the absence of light, with a minimum lethal dose of 100-200 mg/kg upon systemic administration (1,4,10) in mice and rats. Thus, extensive preclinical studies have indicated PCI as a safe and a highly specific *anticancer* treatment; this usually depends on the photosensitizer and light properties used for illumination (1,4-10).

As compared to most other anti-cancer cytotoxic drugs bleomycin has some unusual physico-chemical properties, including hydrophilicity and large size. This makes bleomycin an agent that to a large degree is taken up into cells by endoscytosis, with accumulation in endocytic vesicles severely limiting its activity (14). In accordance with this, preclinical studies have demonstrated that the anti-tumour activity of bleomycin was strongly enhanced using the PCI technology (15,16).

Here we report on the “first-in-man” phase I clinical trial to examine the safety, tolerability and anti-tumour activity of photochemical internalization (PCI) of bleomycin with the photosensitizer $TPCS_{2a}$ (in the treatment of advanced/recurrent cutaneous and subcutaneous malignancies*.*

Materials and Methods

This single-centre, dose-escalation phase I clinical trial was performed at University College Hospital (UCH), London, United Kingdom. The aims of the study were to assess the safety and tolerability of $TPCS_{2a}$-mediated PCI with bleomycin and document the anti-tumour activity of the PCI treatment. This included determining the maximum tolerated dose (MTD), describing the dose-limiting toxicities (DLT), and evaluating body organs toxicity, skin photosensitivity and $TPCS_{2a}$ pharmacokinetics.

This clinical trial was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice Guidelines of the General Medical Council (UK). The trial protocol was approved by the South West Research Ethics Committee, National Health Service, UK (REC Reference Number 09/H0206/17). Every patient was discussed at the multidisciplinary team meeting at UCH and all patients were provided with a detailed information sheet and educated about the treatment and the commitment it required. Each patient signed an informed consent prior to joining the trial. A Dose Escalation Committee reviewed the safety findings of each cohort and agreed on the magnitude of the next dose level escalation.

The patient population included adult patients with advanced and/or recurrent cutaneous or subcutaneous malignancies, with a performance status (PS) of 0-2 on the Eastern Cooperative Oncology Group (ECOG) scale and predicted life expectancy of at least 3 months. For optimal monitoring, patients needed to be of I-IV Fitzpatrick skin type (Table 1). For inclusion in the study, recruited patients needed to be considered safe to receive bleomycin, should have discontinued radiotherapy for at least 2 weeks and chemotherapy for at least 6 half-life cycles prior to the administration of $TPCS_{2a}$. Patients with tumours eroding into a major blood vessel were not recruited into this trial.

A single target area or lesion was identified in each subject on Day -14 (Day 0 reflecting day of $TPCS_{2a}$ administration) and treatment progress of that area was documented. Patients with multiple malignant lesions or areas were assessed and treated. Monitoring of vital signs, ECOG status and for adverse events took place at every scheduled hospital visit. Haematology and biochemistry blood profiling was also undertaken regularly and as per the trial's protocol. Photosensitizer-related assessments were carried out at every visit and included: blood and urine testing for pharmacokinetic analysis, target lesion assessment (response) via clinical photography (aided by ultrasonography and histopathology) and skin photosensitivity testing and scoring (Table 2 and Figure 2).

PCI treatment

On Day 0, $TPCS_{2a}$ was administered by slow intravenous injection into the midcubital vein, with the patient constantly monitored during this process. Dexamethasone and chlorphenamine were administered soon after to reduce any potential allergic reaction effect. Ninety-six hours were allowed to elapse to allow the photosensitiser to distribute in the tumour and be taken up by the tumour cells. . The patient was kept in a dim-light side room to avoid photosensitivity reactions in the skin or the eyes, and monitored closely for adverse events.

On Day 4, bleomycin (fixed dose of 15,000 IU/m^2) was administered by slow intravenous infusion under the supervision of an experienced oncologist. Illumination of the target lesion or area took place three hours (±30 min) later using 652 nm diode laser light. Each illumination process covered a circle of up to 5cm in diameter and lasted 600secs at an irradiance of 100 mW/cm^2, to achieve a fixed light dose of 60 J/cm^2. A margin of 10mm beyond the macroscopic tumour margin was treated to eliminate any micro infiltration. Tissues subjected to the PCI treatment were infiltrated with 10-20ml of 0.5% Bupivacaine (with no vasoconstrictor) as an analgesic. The illumination process was performed with different regimens for pain management (awake, sedation, general anaesthesia) as specified under the results section.

In the immediate post-treatment phase, the patient analgesic requirements were satisfied through special pain protocols. Dose escalating the patient's own pain medication or prescribing patient-controlled analgesics was implemented when indicated. Medical and surgical unwanted events were dealt with immediately. Airway control was a priority (when managing patients with oral/oropharyngeal/laryngeal malignancies) as compromise can occur from the resulting local inflammatory reaction. Elective tracheostomy was implemented in the peri-treatment phase when indicated. Patients were instructed to take precautions to restrict exposure of their skin and eyes to light until otherwise instructed. Patients were discharged on Day 7 when clinically indicated, and were followed up on Day 14, Day 28 and at 3 months from the day of photosensitiser (Amphinex®) administration (Day 0). As part of this trial, each patient received one round of the PCI treatment only. Any other clinically indicated investigations or interventions were implemented without delay and according to the patient's best interests.

Dose escalation, dose-limiting toxicity and maximum tolerated dose

The TPCS2a starting dose was 0.25 mg/kg and was to be escalated in successive dose groups of 3 patients according to a modification of Simon's accelerated titration design (17). Doses were to be doubled until dose-limiting toxicity (DLT - a state where side effects are known to be severe preventing more treatment) was observed in one patient during 28 days' follow-up. Drug-related DLT was defined, recorded according to the Common Terminology Criteria for Adverse Events v3.0 (CTCAE), (Table 1). Since major reactions in the illuminated area would mainly be due to intended destruction of tumour tissue, a higher grade of toxicity was accepted inside this area. If a DLT was observed in one patient in a dose group, subsequent dose levels were to be escalated by 1.5 times until DLT occurred in at least two patients. If more than 33% of the patients at a given dose level report a DLT within the first 28 days of treatment the maximum tolerated dose (MTD) would have been exceeded, and dose escalation would be stopped. MTD was then defined as the dose below. Based on the MTD a further six patients were to be treated at a dose level below the MTD as the principal investigator deemed to be sufficient for the treatment of lesions, (Figure 2).

General and photosensitiser-related assessments

I. Medical examination and investigations

General medical examination was carried out at every patient's visit. Routine blood testing was undertaken regularly to monitor body organs function.

II. Unwanted and adverse events

Peri-treatment medical and surgical unwanted events were identified and dealt with promptly. Adverse events were recorded and reported according to ICH GCP guidelines.

III. Pain scoring and patients' performance status
Pain was scored by the patient on a 10 cm visual analogue scale (VAS) on Day 4 (just after light application) and Day 5.

IV. Blood and urine assessments for Pharmacokinetic (PK) analysis
Blood and urine samples for pharmacokinetic analysis were taken on Days 0 (pre-$TPCS_{2a}$, 30 min and 4 hours post-$TPCS_{2a}$ administration), 2, 4, 7, 14, 28, and at the last visit (Table 2). Content of $TPCS_{2a}$ (Amphinex®) in plasma and urine was analysed by fluorescence spectroscopy.

V. Skin photosensitivity testing and scoring
- Skin photosensitivity was assessed and recorded at specified intervals throughout the trial (Days 0,1,3,6,14,28 and last visit). Skin photosensitivity tests were carried out with white light at two intensities: 500 lux (comparable to bright indoor light) and 100,000 lux (comparable to direct sunlight) for periods ranging of 30 seconds or 5 minutes (Table 2). Separate 0.8 cm^2 spots on the inside of the arm were exposed to light and patients were evaluated at 1 and 24 hours after exposure.

It was considered important to report any local skin changes, as these may reflect phototoxicity, including: erythema, oedema, blister formation, hypopigmentation, hyperpigmentation, scarring, atrophy, induration and skin defects. The scoring of skin photosensitivity was descriptive.

VI. Target lesion assessment response
Target lesion/area measurements by clinical examination (largest diameter), and ultrasonography [US] when applicable, were recorded at Days -14, 0, 28 and last visit. Clinical photography was carried out on Days 0, 7, 14, 28 and last visit (Table 2). Tissue specimens were sent for histopathological analysis, when appropriate, to assess response and tumour margins. Response was recorded according to "Response Evaluation Criteria in Solid Tumours" (RECIST version 1.1) under the categories Complete Response (CR), Partial Response (PR), Stable Disease (SD) or Progressive Disease (PD). Confirmed RECIST responses were those that have been confirmed at Day 28 (Table 1).

Statistical evaluation
Safety and response data were summarised using descriptive statistics by dose level. Statistical analysis comparing the response of the target lesion to the treatment with adverse effects due to photosensitivity was carried out using SPSS (version 20).

Results

In total 22 patients were enrolled in this trial, with the demographics outlined in Table 3. The majority of patients were Caucasians, with a mean age of 60 years and an ECOG of 0 and 1 in 10 and 9 patients, respectively, while 3 patients had ECOG 2. The majority of patients had squamous cell carcinoma of the head and neck but there were also a variety of other advanced and/or recurrent malignancies of the head and neck, torso and upper limbs including sarcoma, eccrine (adnexal) carcinoma and chemo-resistant ductal carcinoma (Fig.1). All patients had a predicted life expectancy of more than 3 months, and had previously received at least 1 surgical treatment for the same treated area along with chemo-radiation. Target lesions were identified, and parameters including tumour depth and longest diameter were recorded (Table 3).

According to the dose escalation plan, patients were divided into the following cohorts: one starting dose (0.25mg/kg), three dose escalations (0.5, 1.0 and 1.5 mg/kg), one "selected optimal dose" with an increased number of patients (0.5mg/kg) and one dose de-escalation (0.125 mg/kg) (Figure 2). The "selected optimal dose" cohort was mainly based on the dose-limiting toxicity (DLT) and maximum tolerated dose (MTD) information, which were actively examined during the trial as well as the therapeutic depth of effect. Sub-group analysis of the $TPCS_{2a}$ dose cohorts is illustrated in table 4.

Twenty-one out of the twenty-two treated patients reached the 4-week follow-up where the DLT and MTD were assessed; all the safety data were also acquired. Only one patient died in the first 4 weeks following the treatment (Figure 1B). Twelve patients completed the clinical trial at the 3-month follow-up. Five out of the ten patients, who left the trial, required further treatment. Two patients suffered from local disease progression and required further intervention and further two suffered from complications of existing metastatic lung disease (Figure 2).

Safety and tolerability of $TPCS_{2a}$-mediated PCI with bleomycin

The administration of $TPCS_{2a}$ was found to be safe and tolerable by all patients. There were no clinically meaningful changes in the vital signs when compared to baseline, furthermore there were no consistent patterns of change over time in mean haematology and biochemistry blood profiles. $TPCS_{2a}$ and the PCI treatment were not found to cause any negative direct effects on any of the monitored body organs. The ECOG performance status was generally unchanged after baseline in 15 patients; while it improved in 2 patients and worsened in 3 patients. Two patients did not have complete ECOG status data.

There were no unwanted or adverse events in the first 96 hours until the administration of the chemotherapeutic agent and the initiation of the PCI treatment. Adverse events have been accurately recorded and classified into 3 categories: (1) PCI-treatment related, (2) Cancer-related, and (3) General medical/mental health-related. PCI-treatment related adverse events were either local or systemic (Table 5). The localised high pain level was the only unexpected event among the PCI-related adverse events. Our clinical observation data suggested that pain was reported after few minutes from initiating the illumination procedure and escalated to maximal levels, then started to decline 1-2 hours later and returned to reasonable (clinically expected) levels at 5-7 hours.

Pain scoring on VAS was highest in the first cohort (0.25 mg/kg) immediately after light delivery (8.08 cm) as they were treated with loco-regional anaesthesia only. All subsequent patients received general anaesthesia or sedation (along with loco-regional anaesthesia), resulting in considerably better pain control reflected in lower VAS scores (Table 6). In the 0.125 mg/kg cohort the VAS score unexpectedly was 7.67 immediately after treatment. In all groups the VAS score was substantially reduced 24 hours after light delivery with mean values ranging from 0.00- 2.38 cm for the different dose groups, with no obvious dose relationship.

Cancer-related adverse events were all expected. Dysphagia was observed due to tumour growth, which was unrelated to the PCI treatment field. Two patients suffered from loco-regional haemorrhage due to tumour invading the blood vessel walls, and the associated fistula formation. General medical and mental health-related adverse events were reported. The severity of adverse events was also recorded. It was found that the majority of PCI-related adverse events (reported more than once) were seen in the cohort receiving photosensitiser ($TPCS_{2a}$) at the dose of 1.5mg/kg (Tables 5 and 6).

Maximum tolerated dose (MTD) and dose-limiting toxicity (DLT)

No adverse events corresponding to the definition of dose limiting toxicity (DLT) were observed in the first two cohorts (0.25 and 0.5 mg/kg). One DLT was seen in the 1.0 mg/kg cohort and the next dose level was therefore 1.5 mg/kg. Two patients (out of the 3) in the 1.5 mg/kg dose cohort experienced DLTs. One of these was a photosensitivity reaction (Grade 3 oedema and Grade 2 blisters to back of hands) 25 days after $TPCS_{2a}$ administration in a patient whose hands were exposed to strong sunlight for a prolonged period, against the protocol recommendations. The DLT in the other patient was a Grade 3 wound infection. These DLTs lead to the conclusion that the maximum tolerated dose (MTD) for $TPCS_{2a}$-based PCI with Bleomycin had been exceeded, and the MTD was therefore agreed to be 1.0 mg/kg.

Pharmacokinetics

The mean plasma concentration of $TPCS_{2a}$ following $TPCS_{2a}$ administration in the four higher dose levels tested is presented in figure 3. In the 0.125 mg/kg dose level, some of the patients were retreated with PCI during the evaluation time, therefore this group could not be included in the pharmacokinetic analysis

The highest mean $TPCS_{2a}$ concentration was observed at the sample point of 30 minutes (0.02 days) after $TPCS_{2a}$ administration. In the 0.25, 0.5 and 1.0 mg/kg dose levels, there was a near proportional relationship between dose and mean maximum concentration, while at the highest dose (1.5 mg/kg) the maximum concentration appeared to have plateaued. After a rapid first phase of elimination, levels of $TPCS_{2a}$ decrease monotonically towards zero through the whole assessment period of 90 days, with the exception of Day 7 in the 1.5 mg/kg dose group, where the $TPCS_{2a}$ level was higher than on Day 4 (Table 7).

The pharmacokinetic behaviour of $TPCS_{2a}$ was further analysed using a non-compartmental approach based on the last four measurements for each patient. There was no significant

difference between the dose levels neither in the mean elimination rates, nor in the mean elimination half-life (ranging from 15.4 to 21.7 days). As expected, the mean values of $AUC_{0-\infty}$ increased with increasing dose, ranging from 47 (µg/mL) x days in the 0.25 mg/kg group to 221 (µg/mL) x days in the 1.5 mg/kg group (Table 7). $TPCS_{2a}$ was still detectable in blood 90 days after administration in all evaluable dose levels.

No $TPCS_{2a}$ was detectable in urine in the first 14 patients; therefore it was decided not to conduct urine analyses in the remaining patients.

Photosensitivity testing and scoring

There were no reported photosensitivity reactions in any patient to exposures of 500 lux (bright indoor light). At 100,000 lux (corresponding to direct sun light), photosensitivity was detected in at least one patient in all dose cohorts except the 0.125 mg/kg. All except one of the reactions were observed between Day 3 and the last visit. All reactions were mild, apart from one patient (1.5 mg/kg dose level) who had moderate (Grade 2) oedema and erythema. Most of the reactions resolved within 24 hours. There appeared to be a correlation between both the frequency and duration of observed photosensitivity reactions and $TPCS_{2a}$ dose.; this is based on qualitative assessment. This last aspect can be exemplified by the observation of skin photosensitivity at Day 90 in the 1.5mg/kg cohort and the absence of photosensitivity reactions beyond Day 14 in the 0.25mg/kg cohort (Figure 4).

Tumour Response

The most striking finding from this trial is the strong tumour responses. The starting dose of $TPCS_{2a}$ was set at a level not expected to trigger a PCI response, however there appeared to be a localized synergistic effect with the photo-activation.

RECIST evaluations were available for target lesions of 16 patients at Day 28 and for 11 patients at their last visit, a minimum of four weeks later. At Day 28, complete response (CR) was achieved in 11 patients (68.8%), partial response (PR) in 2 (12.5%), stable disease (SD) in 2 (12.5%) and progressive disease (PD) in only one patient. Furthermore, the outcome at the last clinic review was complete response (CR) in 5 (45.4%) patients, partial response (PR) in 2 (18.2%), stable disease (SD) in 2 (18.2%) and progressive disease (PD) 2 (18.2%) patients (Table 8). This effect was not only confined to squamous cell carcinomas (Figures 5 and 6) but was also observed in other tumour types such as sarcoma (Figure 7) and chemo-resistant ductal carcinoma (Figures 8 and 9) which have traditionally been very resistant to most treatment modalities.

At 28 days, target lesions had completely resolved in all 4 patients in the 0.25 mg/kg cohort. In the 0.5 mg/kg cohort, 4/7 patients had a CR. As the original dose escalation had demonstrated good efficacy even in the lowest dose level of 0.25 mg/kg (100% CR), it was decided to evaluate the clinical response at a lower dose level of 0.125 mg/kg (dose se-escalation). However, the clinical response in this dose group was inferior to that observed in the higher dose groups and this dose de-escalation group was not included in our RECIST evaluations (Table 8).

Five patients succumbed to their illness, caused by complications of the disease at distant organs. Two with pre-existing lung metastasis died of pulmonary haemorrhage, one poorly

controlled asthma with long standing history of opiate consumption succumbed to multi-organ failure and 2 patients suffered a cardio-respiratory arrest. No PCI-related deaths were recorded.

Target lesion, local tumour-specificity and optimal photosensitiser dose

Identifying a target lesion for every patient included in this trial represented a challenge (Figures 1A, 1B, 1F and 1G). Our data has clearly proven the ability of PCI in eliminating a variety of malignancies, with preservation of adjacent non-malignant tissues (Figures 5- 10).

In patients with cutaneous malignancies, it was observed that after illumination, the malignant area turned necrotic, while the surrounding normal skin, although illuminated, remained intact (Figures 5 Month 3, 6 Day 45, 7 Day 28). Likewise, when a subcutaneous malignancy was illuminated, the cancerous lesion became necrotic (Figures 8 and 10) with no damage to the illuminated healthy overlying skin (Figure 10).

Based on the “target lesion” data, the 0.25mg/kg cohort achieved a complete tumour response to therapy (as per RECIST). However, the overall treatment data (including target lesions and all other treated lesions) have shown that the 0.5mg/kg cohort achieved a higher tumour therapeutic depth. As such, a decision was made to highlight the 0.5mg/kg as the “selected optimal dose” cohort.

Discussion

The new photosensitiser $TPCS_{2a}$ when used in combination with bleomycin was demonstrated to be safe and tolerable by all patients, and was an acceptable treatment option in a very complex population of patients with various cutaneous and/or sub-cutaneous malignancies (at day 28, CR was achieved in 11/16 patients). No PCI-related death was identified in any of the 5 patients who succumbed to their illness during the operation of this trial.

The unexpected high levels of pain experienced during illumination were eliminated by the use of general anaesthesia or intravenous sedation. Post-intervention pain was managed through special pain management protocols, and our data have shown significant reduction in pain levels 24 hours post illumination. This discomfort was localized to the site of the malignancy. Based on clinical observations, the pain appeared to be correlated with surface area of tumour exposed to illumination. We hypothesize that the induction of acute necrosis with the release of intracellular degradation products may have stimulated small pain fibres either directly or through histamine, chemokines and cytokines release (18).

One of the DLTs of $TPCS_{2a}$-mediated PCI was skin photosensitivity. The most severe event seen was at the dose of 1.5 mg/kg in a patient who did not follow the general precautions given to prevent skin sensitivity reactions. Controlled skin photosensitivity measurements indicated that skin photosensitivity was dose dependent, being substantially less at the 0.25 mg/kg dose, which still provided an effective therapeutic response.

Skin photosensitivity was observed for a substantial period of time following $TPCS_{2a}$ administration, particularly at higher doses but was clinically manageable. This prolonged effect was also reflected in the pharmacokinetic measurements, which demonstrated that $TPCS_{2a}$ has a slow elimination rate (half-life 15-22 days) and was still detectable in blood 90 days after administration in all evaluable dose levels. There was no indication that the employment of PCI increases the reported skin toxicities of bleomycin (19), also in accordance with animal studies indicating that the administration of bleomycin did not increase skin photosensitivity over what was seen with $TPCS_{2a}$ and illumination alone (14,16).

A very interesting finding from this trial was the strong tumour responses observed. Although the MTD of $TPCS_{2a}$ was determined to be 1.0 mg/kg, strong anti-tumour effects were observed at all doses from the starting dose of 0.25 mg/kg. Apart from the inferior response observed when the dose was reduced to half the starting dose (de-escalation cohort), there was no obvious correlation between the $TPCS_{2a}$ dose and the tumour response, with seemingly similar effects observed at 0.25 and 1.5 mg/kg. Also good tumour responses were observed across several different tumour types, such as squamous cell carcinomas, sarcoma and chemo-resistant ductal carcinoma, which have traditionally been very resistant to most treatment modalities. The preliminary tumour response results from the present study indicate that a photochemical dose sufficient to produce a complete tumour response can be employed without inducing severe damage to surrounding normal tissue within the illuminated field.

The PCI treatment would appear to induce an immediate but gradual process, with the tumour tissue slowly developing necrosis over a number of weeks. PCI has shown good potential of high tumour selectivity when treating cutaneous and sub-cutaneous malignancies. In contrast and based on clinical observation data, local treatment of similar lesions with photodynamic therapy using the Foscan® photosensitizer induces a process after few days but more rapid tumour necrosis with considerably less tumour specificity.

This suggestion of tumour selectivity was somewhat unexpected, since in mouse xenograft studies the distribution of $TPCS_{2a}$ has not been demonstrated to discriminate well between tumour tissue and adjacent normal skin (16). However, in a hamster cheek pouch model for head and neck cancer, some tumour selectivity of $TPCS_{2a}$ accumulation has been observed (16), and significant selectivity (5-7 times) between tumour tissue and underlying muscle tissue has been observed in mice (20), indicating that some specificity may be due to selective accumulation of $TPCS_{2a}$ in the tumour tissue.

Bleomycin in itself, is not very tumour-selective, however it is more toxic to highly proliferating cells and it is possible that the cellular uptake (21), or the biological effect of bleomycin may be higher in cancer cells than in surrounding normal cells in the skin, although, at least to our knowledge, this has not been formally investigated. The acute dose limiting side effects of bleomycin, including myelosuppression, would not be expected to be increased by a locally directed therapy such as PCI, and this was confirmed to be the case, and cumulative pulmonary toxicity seen with bleomycin (22) is mitigated by PCI treatment being only a single administration.

As well as the tumour response data described, the survival data were encouraging. The mean survival time of all patients studied (excluding the 0.125mg/kg cohort) was 17 months, and several patients with locally recurrent malignancies (without distant metastases) had remarkably long survival, with two patients still being alive more than 4 years after treatment, and additional 4 patients living more than 2 years after treatment. Only two of the long survivors received subsequent chemotherapy and given the mean age and comorbidities in these patients.

PCI targeting disease at molecular level

The Photochemical internalisation (PCI) technology has been developed in various *in vitro* systems and very promising effects have been observed in many animal models. In preclinical studies, it has been found that PCI can enhance the effect of endocytosed molecules even when the target cells are exposed to subtoxic illumination doses (12-14). This predicts that PCI may be able to produce deeper effects than achievable by pure photodynamic therapy (13,23). However, it was rather surprising that in this study complete tumour responses were observed in tumours with a depth of up to 35 mm. The fluence reaching the deepest part of such tumours is exceedingly small, implying that either the light dose needed to induce release of sufficient amounts of Bleomycin is smaller than anticipated, or that mechanisms other than the cytotoxic effect of Bleomycin may be contributing in the deeper layers of the tumour. One possible mechanism is the induction of an immunological response, which has been described for photochemical treatments, and which could also explain the selectivity of the treatment (24).

In addition to the possible immune-stimulating induction of necrosis, inflammation and cytokine production (25), it has recently been reported that PCI can enhance antigen presentation on major histocompatibility complex class I, an important element in the generation of an effective immune response to tumours (26,27). There were no obvious ischaemic effects observed in surrounding normal tissues, which may imply that if blood supply is affected by the treatment it is limited to more fragile tumour neovascularisation.

An interesting aspect of the PCI technology is that $TPCS_{2a}$ and similar molecules, in contrast to many other photosensitisers, are not affected by many of the more common drug resistance mechanisms (28,29). For example, the effect of $TPCS_{2a}$ is not affected by the expression of the ABCG2 transporter, which contributes to drug resistance in highly drug-resistant putative cancer stem cells (30). Thus, PCI has a very interesting potential for treating tumours with acquired drug resistance (31) and perhaps even the inherently chemo-resistant cancer stem cells.

The PCI technology also has potential beyond use with cytotoxic drugs, such as with macromolecular agents. Many macromolecules are totally dependent on endosomal release to reach intracellular targets and therefore have the potential to have greatly enhanced activity when combined with PCI. For example, *in vitro* studies have shown that PCI can enhance the effect of a protein toxin more than 1000-fold (31). This raises the possibility that PCI may be able to cause 'release' of the toxins for a cytotoxic effect in the illuminated target area at doses where there is no effects in other (non-illuminated) parts of the body. A particularly interesting option is to combine PCI with antibody-based molecules, such as immunotoxins or antibody drug conjugates with intracellular targets. Thus, *in vitro* studies have shown that PCI can enhance the effect of immunotoxins directed to several different relevant cancer surface markers such as the EGFR (30, 32) and others (33,34), and *in vivo* data has also shown very potent and specific effects of PCI with a recombinant immunotoxin directed to a cell surface marker that is overexpressed on melanoma and several other cancer types (11).

Conclusion

The very promising results of the $TPCS_{2a}$-mediated photochemical internalisation (of chemotherapeutic agent bleomycin) in this heavily pretreated population suggest it may have an important role in interventional oncology.

It was very encouraging to observe the uniform effect of PCI in causing tumour death on a number of very aggressive cutaneous and sub-cutaneous malignancies including squamous cell carcinoma, sarcoma, eccrine (adnexal) carcinoma and chemo-resistant ductal carcinoma. The study paves the way for further clinical development of the PCI/ $TPCS_{2a}$/Bleomycin combination, as well as clinical testing of the technology with other types of drug molecules on different tumour types.

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Conflict of interests/Competing interests

Anders Høgset: Works as Chief Scientific Officer for PCI Biotech AS

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Tables

Table 1: The parameters and their sub-classifications employed for patient's inclusion criteria, evaluation and assessment of outcome.

Fitzpatrick skin type	
Type I Pale white	**Type IV** Moderate brown
Type II White	**Type V** Dark brown
Type III Cream white	**Type VI** Deeply pigmented dark brown to black
ECOG	
0 Fully active	**3** Capable of only limited self-care
1 Restricted in physically strenuous activity	**4** Completely disabled
2 Ambulatory and capable of all self-care	**5** Dead
CTCAE	
(i) Photosensitivity Grade 2 outside the illuminated area, except for areas exposed for skin sensitivity tests and areas wilfully exposed for re-introduction to normal light.	
(ii) Photosensitivity Grade 4 inside the illuminated area.	
(iii) Neutropenia or thrombocytopenia Grade 4.	
(iv) All other toxicity reactions (excluding nausea and vomiting) of ≥ Grade 3.	
RECIST criteria	
Complete response (CR): Disappearance of all target lesions.	
Partial response (PR): At least a 30% decrease in the sum of the longest diameter (LD) of target lesions taking as reference the baseline sum LD. Confirmed at 4 weeks.	
Stable disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD taking as references the smallest sum LD.	
Progressive disease (PD): At least a 20% increase in the sum of LD of target lesions taking as references the smallest sum LD recorded since the treatment started or the appearance of one or more new lesions.	

Table 2: The peri-PCI treatment visits highlighting the required assessments and procedures.

	Pre-study	**Baseline**	**Visit 1**	**Visit 2**	**Visit 3**	**Visit 4**	**Visit 5**	**Visit 6**	**Visit 7**	**Visit 10**	**Visit 14**	**Visit 16**
	Day -14	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 14	Day 28	3 months
Medical assessment	✓	✓				✓				✓	✓	✓
Performance status	✓	✓									✓	✓
Bloods test	✓	✓				✓				✓	✓	✓
Pharmacokinetics		✓		✓		✓			✓	✓	✓	
Lesion measurement		✓									✓	✓
Clinical Photography		✓							✓	✓	✓	✓
$TPCS_{2a}$		✓										
Bleomycin given						✓						
Laser treatment						✓						
Pain assessment						✓	✓					
Skin photo testing		✓	✓		✓			✓		✓	✓	
Skin photo score		✓	✓	✓	✓	✓		✓	✓	✓	✓	

Table 3: Patients demographics, target lesions and Amphinex dosing parameters.

	Patients		**Patients**
	No. = 22		No. = 22
Gender		**Target lesion (TL) location**	
Male	11	Head and neck	17
Female	11	Torso – front	3
		Torso – back	1
Age (at time of treatment)		Arm	1
Mean±SD	60.4±13.3		
Minimum-Maximum	34-82	**TL – longest diameter** (mm)	
		Mean ±SD	38 ±21.5
Race		Minimum-Maximum	15-120
Caucasian	20		
Asian	2	**TL – depth** (mm)	
		Mean ±SD	16.0 ±9.5
Fitzpatrick skin type		Minimum-Maximum	2-38
Type I	4		
Type II	7	**Trial cohorts**	
Type III	8	Starting dose	1
Type IV	3	Dose escalation	3
		Dose de-escalation	1
ECOG		Optimal photosensiter	1
0	10		
1	9	**Trial cohorts – dosing mg/kg**	
2	3	0.125	3
		0.25 (starting dose)	4
Diagnosis		0.5 (selected photosensitizer)	9
Squamous cell carcinoma	16	1.0	3
Sarcoma	1	1.5	3
Ductal carcinoma	4		
Eccrine (Adnexal) carcinoma	1		

Table 4: Patients demographics and target lesion parameters as per $TPCS_{2a}$ dosing groups.

	0.125 mg/kg	**0.25 mg/kg**	**0.5 mg/kg**	**1.0 mg/kg**	**1.5 mg/kg**
Patients No.	3	4	9	3	3
Gender					
Male	2 (67%)	4 (100%	2 (22%)	2 (67%)	1 (33%)
Female	1 (33%)	0 (0%)	7 (78%)	1 (33%)	2 (67%)
Age (at time of treatment)					
Mean ±SD	58.0 ±7.0	69.8 ±12.1	56.4 ±12.8	49.7 ±19.1	72.0 ±7.8
Minimum-Maximum	52-65	56-82	40-73	34-72	67-81
Race					
Caucasian	3 (100%)	4 (100%)	8 (89%)	2 (67%)	3 (100%)
Asian	0 (0%)	0 (0%)	1 (11%)	1 (33%)	0 (0%)
Fitzpatrick skin type					
Type I	0 (0%)	2 (50%)	2 (22%)	0 (0%)	0 (0%)
Type II	0 (0%)	0 (0%)	4 (45%)	1 (33%)	2 (67%)
Type III	3 (100%)	2 (50%)	2 (22%)	0 (0%)	1 (33%)
Type IV	0 (0%)	0 (0%)	1 (11%)	2 (67%)	0 (0%)
ECOG					
0	1 (33%)	2 (50%)	6 (67%)	0 (0%)	1 (33%)
1	2 (67%)	1 (25%)	3 (33%)	2 (67%)	1 (33%)
2	0 (0%)	1 (25%)	0 (0%)	1 (33%)	1 (33%)
Diagnosis					
Squamous cell carcinoma	3 (100%)	3 (75%)	7 (77.8%)	2 (67%)	1 (33%)
Sarcoma	0 (0%)	1 (25%)	0 (0%)	0 (0%)	0 (0%)
Ductal carcinoma	0 (0%)	0 (0%)	2 (22.2%)	1 (33%)	1 (33%)
Eccrine (Adnexal) carcinoma	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (33%)
Target lesion (TL) location					
Head and neck	3 (100%)	4 (100%)	6 (66.7%)	2 (67%)	2 (67%)
Torso – front	0 (0%)	0 (0%)	2 (22.2%)	0 (0%)	1 (33%)
Torso – back	0 (0%)	0 (0%)	1 (11.1%)	0 (0%)	0 (0%)
Arm	0 (0%)	0 (0%)	0 (0%)	1 (33%)	0 (0%)
TL – longest diameter (mm)					
Mean ±SD	30.3±17.0	48.8 ±47.7	34.6 ±11.4	48.3 ±2.9	31.3 ±2.3
Minimum-Maximum	20-50	20-120	15-55	45-50	30-34
TL – depth (mm)					
Mean ±SD	16.3 ±11.8	17.5 ±9.5	14.3 ±8.2	23.3 ±15.6	11.0 ±3.6
Minimum-Maximum	9-30	4-25	2-25	7-38	8-15

Table 5: The adverse events (observed more than once per patient) and as per severity. The adverse events have been classified into PCI-related (directly linked to PCI treatment), cancer-related (directly linked to the natural progression of the malignancy) and general medical/mental health related (directly linked to the patient's general medical and mental health). Each event was highlighted according to the clinical judgment of the treating team as either being an "expected" event or "unexpected" event.

Adverse event	**Expected event or not**	**Incidence (No. = 22)**
Mild adverse events: transient and easily tolerated.		15 patients
Moderate adverse events: causes the patient discomfort and interrupts the patient's usual activities.		10 patients
Severe adverse events: causes considerable interference with the patient's usual activities, and may be incapacitating or life-threatening.		14 patients
PCI-related (observed more than once)		
Localised pain	**Unexpected**	**9 patients**
Localised erythema	Expected	3 patients
Localised swelling	Expected	3 patients
Localised infection	Expected	2 patients
Localised sensory disturbance	Expected	4 patients
Nausea and vomiting	Expected	6 patients
Photosensitivity skin reaction - simple	Expected	3 patients
Photosensitivity skin reaction - pruritus	Expected	3 patients
Cancer-related (observed more than once)		
Dysphagia	Expected	2 patients
Local haemorrhage	Expected	2 patients
Fistula formation	Expected	2 patients
Death	Expected	5 patients
General medical/mental health-related (observed more than once)		
Dyspepsia	Expected	2 patients
Drowsiness	Expected	2 patients
Constipation	Expected	3 patients
Fatigue	Expected	2 patients
Respiratory failure	Expected	2 patients
Panic attack	Expected	2 patients

Table 6: The incidence and severity of adverse events are shown for the different $TPCS_{2a}$ dosing groups.

	0.125 mg/kg	**0.25 mg/kg**	**0.5 mg/kg**	**1.0 mg/kg**	**1.5 mg/kg**
Number of patients	3	4	9	3	3
Total adverse events[1]	9	6	47	7	35
PCI-related adverse events[1]	4	5	10	1	24
Total PCI-related adverse events[1] (observed once or more)					
Mild	4 (100%)	2 (40%)	4 (40%)	1 (100%)	19 (79%)
Moderate	0 (0%)	0 (0%)	2 (20%)	0 (0%)	3 (13%)
Severe	0 (0%)	3 (60%)	4 (40%)	0 (0%)	2 (8%)
PCI-related pain scoring (in cm on VAS)					
Immediately after illumination	7.67	8.08	4.28	1.70	5.05
24 hours after illumination	2.47	1.45	2.62	0.00	1.72
PCI-related adverse events[2] (observed more than once)					
Mild	2 (100%)	2 (40%)	4 (40%)	1 (100%)	11 (68.8)
Moderate	0 (0%)	0 (0%)	2 (20%)	0 (0%)	3 (18.7)
Severe	0 (0%)	3 (60%)	4 (40%)	0 (0%)	2 (12.5)
Cancer-related adverse events[2] (observed more than once)					
Mild	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Moderate	0 (0%)	0 (0%)	4 (50%)	2 (67%)	0 (0%)
Severe	0 (0%)	0 (0%)	4 (50%)	1 (33%)	0 (0%)
General medical/mental health-related adverse events[2] (observed more than once)					
Mild	1 (33%)	0 (0%)	3 (50%)	0 (0%)	0 (0%)
Moderate	2 (67%)	1 (100%)	2 (33%)	1 (50%)	1 (100%)
Severe	0 (0%)	0 (0%)	1 (17%)	1 (50%)	0 (0%)

[1]Total adverse events considered possibly or probably related (observed once or more). Patients could have AEs of more than one severity or intensity.

[2]Total adverse events considered possibly or probably related (observed more than once). Patients could have AEs of more than one severity or intensity.

Table 7: The concentration of $TPCS_{2a}$ in patient plasma samples was measured by fluorescence spectroscopy.

	0.25 mg/kg	**0.5 mg/kg**	**1.0 mg/kg**	**1.5 mg/kg**
Number of patients	4	9	3	3
Maximum concentration of $TPCS_{2a}$ in plasma (µg/mL)				
Mean ±SD	5.7 ±1.2	10.3 ±1.3	16.9 ±3.0	18.2 ±2.6
Minimum-Maximum	4.2–6.8	8.6–12.1	13.5–19.4	15.5–20.6
Daily elimination rate of $TPCS_{2a}$ in plasma				
Mean ±SD	0.036 ±0.004	0.040 ±0.074	0.046 ±0.074	0.033 ±0.006
Minimum-Maximum	0.033–0.041	0.031–0.055	0.038–0.052	0.027–0.038
Elimination half–lives of $TPCS_{2a}$ in plasma (days)				
Mean ±SD	19.6 ±1.9	18.0 ±3.0	15.4 ±2.6	21.7 ±3.7
Minimum-Maximum	16.8–21.1	12.7–22.6	13.2–18.3	18.0-25.5
$AUC_{0-\infty}$ of $TPCS_{2a}$ in plasma (µg/mLxdays)				
Mean ±SD	47 ±10	76 ±18	114 ±32	221 ±50
Minimum-Maximum	40–60	49–100	81–146	183–277

Table 8: Investigator evaluation of response of the target lesion. The evaluation of the response of the target lesion to the treatment is shown for the safety and the per protocol populations. The responses were evaluated according to the RECIST criteria.

	0.25 mg/kg	**0.5 mg/kg**	**1.0 mg/kg**	**1.5 mg/kg**
Total number of patients included	**4**	**9**	**3**	**3**
Number of patients (Day 28)	4	7	2	3
Number of patients (Last review)	2	6	1	2
Day 28 (19 patients)				
Complete response	4 (100%)	4 (44%)	2 (67%)	1 (33%)
Partial response	0 (0%)	0 (0%)	0 (0%)	2 (67%)
Stable disease	0 (0%)	2 (22%)	0 (0%)	0 (0%)
Progressive disease	0 (0%)	1 (11%)	0 (0%)	0 (0%)
Missing	0 (0%)	2 (22%)	1 (33%)	0 (0%)
Last visit (11 patients)				
Complete response	2 (100%)	2 (33%)	0 (0%)	1 (50%)
Partial response	0 (0%)	1 (17%)	0 (0%)	1 (50%)
Stable disease	0 (0%)	2 (33%)	0 (0%)	0 (0%)
Progressive disease	0 (0%)	1 (17%)	1 (100%)	0 (0%)

Figures

Figure 1: Sample of the palliative patients treated under the phase I PCI trial protocol. All disease progression described occurred prior to the PCI treatment. (A) 56-year-old male with chondroblastic osteosarcoma in the right mandible. He failed 3 surgical resections with reconstruction and chemo-radiation. (B) 45-year-old male with squamous cell carcinoma of the neck. He failed 3 surgical resections with reconstruction and chemo-radiation. The primary cancer also metastasised to the lungs and liver. This is the only patient who succumbed to his illness in his first 4 week on the trial. (C) 61-year-old female with metastatic squamous cell carcinoma (SCC) to the torso (back). The primary malignancy was a tongue base SCC with metastasis to the cervical and axillary lymph nodes. Had multiple failed interventions to the primary site. (D) 46-year-old female with metastatic ductal carcinoma to the torso (anterior). The primary breast cancer also metastasised to the brain, spine, lungs and liver. (E) 72-year-old female with metastatic (chemo-resistant) ductal carcinoma to the arm. The primary breast cancer also metastasised to the axillary and cervical lymph nodes. (F) 35-year-old male with squamous cell carcinoma of the floor of mouth and neck. This patient failed surgery, chemo-radiation and photodynamic therapy. The cancer metastasised to the right lung and required pneumonectomy. (G) 73-year-old male with oral squamous cell carcinoma. This patient failed multiple surgical interventions with reconstruction, as well as chemo-radiation.

Figure 2: Top diagram: Flow chart of the trial progress: initial recruitment, follow-up at Day 28, follow up after Day 28 and last review (Days 78-122). Bottom diagram: the process of dose escalation, de-escalation and fixation at the potential optimal dose, also included are the number of patients per cohort.

Figure 3: The new photosensitiser $TPCS_{2a}$ (Amphinex) pharmacokinetics in blood. The concentration of $TPCS_{2a}$ in plasma samples from patients in the dose cohorts 0.25 – 1.5 mg/kg was analysed by fluorescence spectroscopy.

Figure 4: Photosensitivity test results. The percentage of patients who experienced a response to the photosensitivity test (the most severe response observed at any time point) is shown for the dose groups 0.25-1.5 mg/kg (at 0.125 mg there were no responses). The erythema responses to 5 min 100 000 lux illumination are shown. Oedema was only observed in two patients in the 1.5 mg/kg dose cohort (1 mild and 1 moderate). Also the device to deliver the light for photosensitivity testing is shown.

Figure 5: 61-year-old female with metastatic squamous cell carcinoma (SCC) to the torso (back). The primary malignancy was a tongue base SCC with metastasis to the cervical and axillary lymph nodes. The patient had multiple failed interventions to the primary site. The left hand column images shows the shielding around the lesion after including 10mm of macroscopically healthy looking tissue within the treatment field to eliminate any chance of micro-infiltration. Surface illumination with 652nm diode laser was applied to activate the PCI process ($TPCS_{2a}$ dose 0.5mg/kg). The right hand column images showing the cancerous lesions on Day -14 and Day 28 where complete tumour death (confirmed by histopathology) can be seen without any serious effect on the non-malignant surrounding tissue. Month 3 image shows on-going healing process. Surgical biopsies have been acquired from the centre of ulceration and were found tumour-free.

Figure 6: This 83-year-old Caucasian male was diagnosed with squamous cell cancer of the right face and scalp. The rapidly growing aggressive tumour overcame all previous interventions including a major ablative resection with reconstruction, followed by chemoradiation. The 2 cancerous islands of the right face have extended to involve the whole right face in a short period of time (Day -28 to Day -7). The “target lesion” represented an extensively eroding tumour mass of the right face (Day

0), ($TPCS_{2a}$ dose 0.25mg/kg). Post illumination Day 4 was associated with immediate changes in tissue colour (redness) with transudate/exudates (yellow in colour) leakage from the face; also this was associated with periorbital swelling. Day 7 was associated with further clinical changes (blanching of tissue) which could be attributed to the bleomycin release. The subsequent days following (Days 14-28) were associated with marked tissue changes and tumour shrinkage (necrosis). The complete response of the treated area could be clearly seen following the surgical removal of the dead tissue on Day 40. All the illuminated areas have also responded favourably to the treatment, including the right temporo-parietal-occipital region. Further tumour areas not treated by our limited non-interstitial protocol included tumour deeper to right temporal region and right supraorbital area.

Figure 7: This 56-year-old Caucasian male was diagnosed with chondroblastic osteosarcoma in the right mandible. Initially the patient underwent surgical resection with free tissue transfer reconstruction, followed by chemoradiation. The rapidly growing aggressive tumour overcame all previous interventions and most recently two major ablative resections with reconstruction. The remaining bulk of the later flap can be seen at the bottom of each image. The tumour has eroded through the cheek resulting in a large fistula (Day -10) and continues to grow at high and unusual rate. The "target lesion" was an exophytic tumour mass at the junction of the right oral commissure and the remaining pectoralis major flap inferiorly; 0.25mg/kg $TPCS_{2a}$ was administered on Day 0. Surface illumination based photochemical internalization (PCI) was implemented on Day 4 and all tumour area was subjected to surface illumination; this phase was associated with immediate changes in tissue colour (blanching). Further clinical changes (indicative of tissue necrosis) could be seen on Days 7, 10 and 14. On Day 14 a fraction of the titanium plate became visible. The "target lesion" changed colour similarly to the adjacently treated tissues and disintegrated and could not be seen on Day 21. All the illuminated areas have also responded favourably to the treatment. This includes tumour anterior to the tragus, superior aspect of the remaining pectoralis major flap and the tumours masses growing above the titanium plate (Days 21 and 28). Further tumour areas not treated by our limited non-interstitial protocol included tumour deeper to the illuminated area, posterolateral tongue, tongue base and recurred tumour inside the bulk of the pectoralis major flap. The patient left the trial on day 45 to receive further life-saving interventions.

Figure 8: (A) Ultrasonography images of a 48-year-old female with heterogeneous subcutaneous ductal carcinoma at the level of the left sternal border (Day -14) with Doppler signal demonstrated within it, and no cortical irregularity or erosion to the underlying hard tissue structures were detected. The Day 28 image revealed no recordable Doppler flow in the heterougeneous soft tissue and normal appearance to the underlying bony structures. Biopsies acquired from the soft tissue and bone was tumour free. ($TPCS_{2a}$ dose 0.5mg/kg); (B) Ultrasonography images of a 67-year-old male diagnosed with right glosso-tonsillar fold squamous cell carcinoma (Day -14). The carcinoma was non-existent on Day 28 and biopsies confirmed tumour-free region. ($TPCS_{2a}$ dose 0.25mg/kg).

Figure 9: (A) Histopathology images acquired from a78-year-old male with squamous cell carcinoma of the face on Day 28. Both sections of the tumour margin and the necrotic mass revealed no viable tumour cell. ($TPCS_{2a}$ dose 0.25mg/kg); (B) Histopathology image of an US-guided core biopsy (Day 28) of a 67-year-old male diagnosed with right glosso-tonsillar fold squamous cell carcinoma. The biopsy showed no viable tumour cells. ($TPCS_{2a}$ dose 0.25mg/kg).

Figure 10: Clinical images of a 48-year-old female with subcutaneous ductal carcinoma at the level of the left sternal border. The skin overlying the carcinoma was tumour-free and light illumination was delivered through the skin to the underlying cancerous tissue. Comparing images of Day -14 to Day 28 revealed no damage to the skin confirming the high specificity of the PCI treatment. The US images are shown in Figure 8A.