Disulfonated tetraphenyl chlorin ($TPCS_{2a}$)-induced photochemical internalisation of bleomycin in patients with solid malignancies: a phase 1, dose-escalation, first-in-man trial

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Summary

Background Photochemical internalisation, a novel minimally invasive treatment, has shown promising preclinical results in enhancing and site-directing the effect of anticancer drugs by illumination, which initiates localised chemotherapy release. We assessed the safety and tolerability of a newly developed photosensitiser, disulfonated tetraphenyl chlorin ($TPCS_{2a}$), in mediating photochemical internalisation of bleomycin in patients with advanced and recurrent solid malignancies.

Methods In this phase 1, dose-escalation, first-in-man trial, we recruited patients (aged ≥18 to <85 years) with local recurrent, advanced, or metastatic cutaneous or subcutaneous malignancies who were clinically assessed as eligible for bleomycin chemotherapy from a single centre in the UK. Patients were given $TPCS_{2a}$ on day 0 by slow intravenous injection, followed by a fixed dose of 15 000 IU/m² bleomycin by intravenous infusion on day 4. After 3 h, the surface of the target tumour was illuminated with 652 nm laser light (fixed at 60 J/cm²). The $TPCS_{2a}$ starting dose was 0·25 mg/kg and was then escalated in successive dose cohorts of three patients (0·5, 1·0, and 1·5 mg/kg). The primary endpoints were safety and tolerability of $TPCS_{2a}$; other co-primary endpoints were dose-limiting toxicity and maximum tolerated dose. The primary analysis was per protocol. This study is registered with ClinicalTrials.gov, number NCT00993512, and has been completed.

Findings Between Oct 3, 2009, and Jan 14, 2014, we recruited 22 patients into the trial. 12 patients completed the 3-month follow-up period. Adverse events related to photochemical internalisation were either local, resulting from the local inflammatory process, or systemic, mostly as a result of the skin-photosensitising effect of $TPCS_{2a}$. The most common grade 3 or worse adverse events were unexpected higher transient pain response (grade 3) localised to the treatment site recorded in nine patients, and respiratory failure (grade 4) noted in two patients. One dose-limiting toxicity was reported in the 1·0 mg/kg cohort (skin photosensitivity [grade 2]). Dose-limiting toxicities were reported in two of three patients at a $TPCS_{2a}$ dose of 1·5 mg/kg (skin photosensitivity [grade 3] and wound infection [grade 3]); thus, the maximum tolerated dose of $TPCS_{2a}$ was 1·0 mg/kg. Administration of $TPCS_{2a}$ was found to be safe and tolerable by all patients. No deaths related to photochemical internalisation treatment occurred.

Interpretation $TPCS_{2a}$-mediated photochemical internalisation of bleomycin is safe and tolerable. We identified $TPCS_{2a}$ 0·25 mg/kg as the recommended treatment dose for future trials.

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Introduction

Photochemical internalisation 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, and 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 and are therefore unable to exert their therapeutic potential.[1] Photochemical internalisation aims to overcome this hurdle by the use of 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, thereby releasing the contents into the cytosol and allowing the drugs to reach their targets. By site-directed illumination, photochemical internalisation can be used to target drugs preferentially to tumour sites, reducing side-effects in distant normal tissues. The mechanism and practical application of photochemical internalisation was initially described in preclinical models by Berg and colleagues[2] in 1999, highlighting its potential clinical usefulness in delivering cancer therapy, gene therapy, and vaccination.

Results from an in-vitro investigation showed that photochemical internalisation can enhance cellular uptake of chemotherapeutic agents, such as bleomycin, especially those that do not easily cross cellular membranes.[3,4] In-vivo studies[5,6] of photochemical

Research in context

Evidence before this study

We searched PubMed for articles published in English between Jan 1, 1990, and April 1, 2016, using the terms "photochemical internalization (PCI)", "solid malignancies", "bleomycin", "disulfonated tetraphenyl chlorin ($TPCS_{2a}$)", and "clinical trial". We found no clinical trial involving the use of disulfonated tetraphenyl chlorin ($TPCS_{2a}$)-mediated photochemical internalisation in human beings. To our knowledge, this trial is the first study to assess the safety and tolerability of $TPCS_{2a}$-mediated photochemical internalisation of bleomycin in human patients and to document preliminary antitumour activity.

Added value of this study

Photochemical internalisation treatment with $TPCS_{2a}$ was safe and tolerated by all patients. Substantial antitumour effects were seen with all doses tested in patients with different types of solid malignancies, including squamous cell carcinoma, sarcoma, ductal carcinoma, and eccrine (adnexal) carcinoma.

Implications of all the available evidence

Photochemical internalisation could be used for the treatment of all solid tumours, especially chemoresistant tumours. This treatment is likely to be highly suitable for early-stage cancers, as a neoadjuvant to conventional interventions.

internalisation in animal models have analysed various therapeutic agents, variables (eg, drug–light interval and tumour type), and their outcomes, including tumour response, tumour selectivity, and immunological response.[4,7] Photochemical internalisation showed a synergistic effect when combined with radiotherapy or after surgery in mouse xenograft models of human cancer.[8,9]

Both in-vitro and in-vivo models have shown that photochemical internalisation enhances the effect of many types of macromolecules[1,2,5,6,10–15] and also of some small molecule anticancer drugs.[4,16] The photosensitisers used in photochemical internalisation have no serious toxic effects in the absence of light, with a minimum lethal dose of 100–200 mg/kg upon systemic administration in mice[1] and rats.[17,18] Thus, extensive preclinical studies have indicated that photochemical internalisation could be a safe and highly specific anticancer treatment.[1,5,6,10–15]

By contrast with most other anticancer cytotoxic drugs, the chemotherapeutic bleomycin has some unusual physicochemical properties, including hydrophilicity and large size (1·4 kDa). These properties make bleomycin an agent that is largely taken up into cells by endocytosis, with accumulation in endocytic vesicles severely restricting its activity.[3] In accordance with these characteristics, preclinical studies showed that the antitumour activity of bleomycin was strongly enhanced by photochemical internalisation.[4,7]

Although most preclinical studies of photochemical internalisation involved the use of aluminium phthalocyanine disulfonate ($AlPcS_{2a}$) as the photosensitiser, its large number of isomers and its batch-to-batch ratio variations make it unsuitable for standard clinical use. Disulfonated tetraphenyl chlorin ($TPCS_{2a}$; Amphinex, PCI Biotech AS, Oslo, Norway) was therefore developed by di-imide reduction of disulfonated tetraphenyl porphine ($TPPS_{2a}$). Synthesis of $TPCS_{2a}$ results in only three isomers, with low batch-to-batch variations.[7] The salt form of $TPCS_{2a}$ is dimonoethanolamine-$TPCS_{2a}$, whereas the $TPCS_{2a}$ formulation is 30 mg/mL, 10% Cremophor ELP (BASF, Germany) in water.

See Online for appendix

Here we report, to our knowledge, the first phase 1 clinical trial to examine the safety and tolerability of $TPCS_{2a}$-mediated photochemical internalisation of bleomycin, and document the antitumour activity of this treatment in patients with advanced or recurrent cutaneous and subcutaneous malignancies.

Methods

Study design and participants

This single-centre, dose-escalation, phase 1 clinical trial was done at University College Hospital (UCH), London, UK. All participants were enrolled and recruited from the Greater London urban area. Eligible patients were adults (aged ≥18 to <85 years) with advanced, metastatic, or recurrent cutaneous or subcutaneous malignancies (squamous cell carcinoma, ductal carcinoma [cutaneous breast metastases], eccrine carcinoma, and sarcoma), with Eastern Cooperative Oncology Group (ECOG) performance status of 0–2 and predicted life expectancy of at least 3 months. Each patient included in the study had at least one surgical intervention along with chemotherapy and radiotherapy; this was not an inclusion criteria but was expected because all patients had advanced or recurrent disease. Patients with cardiovascular comorbidities (eg, hypertension and ischaemic heart disease) and respiratory comorbidities (eg, asthma and chronic obstructive pulmonary disease) were eligible, as were patients with metastatic disease to the lungs, liver, and spine. Non-permitted comorbidities were musculoskeletal and neurological disorders (eg, motor neuron disease and multiple sclerosis), which can be disabling and require a high level of pain relief. We also excluded patients with metabolic and hormonal disorders because these disorders might affect drug metabolism, elimination, or effects. For optimal monitoring, patients needed to have a Fitzpatrick skin type (a numerical classification system for human skin colour ranging from I [skin that always burns in the sun] to V [skin that tans very easily]) of I–IV (appendix p 1). For inclusion in the study, patients needed to be clinically assessed as

medically fit to receive bleomycin chemotherapy according to our consultant oncologists (DC and MF), and should have discontinued radiotherapy for at least 2 weeks and chemotherapy for at least six half-life cycles before administration of $TPCS_{2a}$. We excluded patients who had received previous photodynamic therapy, those undergoing another type of treatment for the same cancer, and those with porphyria or other diseases exacerbated by light, hypersensitivity to photosensitisers, tumours known to be eroding into major blood vessels or major vessels adjacent to the illumination site, a planned surgical procedure within the next 30 days, and coexisting ophthalmic disease likely to require slit-lamp examination.

This trial was done 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. Every patient was discussed at the multidisciplinary team meeting at UCH and all patients provided written informed consent.

Procedures

On day 0, $TPCS_{2a}$ at the starting dose of 0·25 mg/kg was given by slow intravenous injection (1–6 min) into the median cubital vein, with the patient monitored constantly during this process. Dexamethasone (1 mg intravenously) and chlorphenamine (10 mg intravenously) were given soon afterwards to reduce any potential allergic reaction effect. We allowed 96 h for the photosensitiser to be distributed in the tumour and taken up by the tumour cells. The patient was kept in a dimly lit side room to avoid photosensitivity reactions in the skin or the eyes, and monitored closely for adverse events. The $TPCS_{2a}$ starting dose (0·25 mg/kg) was to be escalated in successive dose groups of three patients according to a modification of Simon's accelerated titration design.[19] This was a modification from our original protocol (for which we planned to have six patients at each dose level), and was made as a result of the high level of clinical activity and low number of treatment-related adverse events, and the difficulty in recruiting patients. This modification was reviewed and accepted by the ethics committee.

Doses were to be doubled until a dose-limiting toxicity (ie, a toxicity regarded as unacceptable by the patient) was recorded in one patient during 28 days' follow-up. If a dose-limiting toxicity was recorded, subsequent dose levels were to be escalated at 1·5 times the preceding dose level until a treatment-related grade 2 toxicity was noted in at least two patients. If a treatment-related grade 2 toxicity was noted in at least two patients during treatment, dose escalation was to proceed at 1·3 times the previous dose level until a dose-limiting toxicity was identified. If the first treatment-related toxicity was grade 3 or worse, the dose level was to be escalated at 1·3 times the preceding dose (rather than at 1·5 times). No dose of $TPCS_{2a}$ could exceed 3 mg/kg bodyweight. If more than 33% of patients at a given dose reported a dose-limiting toxicity within the first 28 days of treatment, the maximum tolerated dose would have been exceeded and dose escalation would be stopped. The maximum tolerated dose was then defined as the dose below that. A further six patients were to be treated at a dose below the maximum tolerated dose; the dose was to be chosen by the principal investigator on the basis of effective tumour therapeutic depth (depth of necrosis) and minimal treatment-related adverse events. Dose reductions or interruptions were not allowed by the protocol.

The dose-escalation cohorts were created after discussions between the investigators and the sponsor regarding assessments for primary and secondary endpoints at each dose. A dose-escalation committee reviewed the safety findings of each cohort and agreed on the magnitude of the next dose escalation. The committee held a teleconference each time the results from one cohort were known. According to the protocol, the data for each cohort was to be final and the database locked for that cohort at the time that the dose-escalation decision was made. However, this was not always practical, and the decision was made when sufficient safety data were available. The committee could decide to revise the dose escalation described in the protocol on the basis of the occurrence of side-effects, pharmacokinetic results, and efficacy results. Additionally, one dose de-escalation cohort was created after a discussion between the investigators and the funder, and was created after completing all the other dose cohorts. The theory was that effective outcomes were achieved with low doses of $TPCS_{2a}$ (ie, 0·25 mg/kg), hence de-escalation was needed to try to identify the subtherapeutic dose, which will be essential for future studies when monitoring $TPCS_{2a}$ concentrations in blood during treatment.

To maximise the treatment effect of photochemical internalisation, the photosensitiser needs to be localised preferentially in the endocytic vesicles of the target cells. In-vivo preclinical studies in mice have shown that the photosensitiser is mostly localised on the plasma membrane 1 day after administration, but not at detectable levels until 48 h after administration.[5] Furthermore, fluorescence whole-body imaging in mice (using the fluorescent properties of the photosensitiser) have suggested that the ratio of tumour to normal tissue of photosensitiser localisation is optimal 3–4 days after photosensitiser administration (Berg K, Oslo University Hospital, Oslo, Norway, unpublished). We have further noted that in-vivo treatment 72 h after photosensitiser administration induces less severe oedema than after 48 h (Berg K, unpublished). Thus, preclinical studies in mice are routinely done with a drug–light interval of 3 days with $TPCS_{2a}$ as photosensitiser.[7] Taking into account the slower pharmacokinetics in human beings than in mice, a decision was made to use a drug–light interval of 4 days.

For the **protocol** see https://www.ucl.ac.uk/surgery/research/imcs/pdfs/PCI_Protocol.pdf

On day 4, bleomycin (15 000 IU/m²) was given by slow intravenous infusion under the supervision of an experienced oncologist. Illumination of the target lesion or area took place 3 h later (give or take 30 min) using 652 nm diode laser light. Each illumination process covered a circle of up to 5 cm in diameter and lasted 600 s at an irradiance of 100 mW/cm², to achieve a fixed light dose of 60 J/cm². A margin of 10 mm beyond the macroscopic tumour margin was treated to eliminate any infiltration of cancer cells into the tumour microenvironment. Before illumination, tissues that were to be subjected to photochemical internalisation treatment were injected with 10–20 mL of 0·5% bupivacaine (with no vasoconstrictor) as an intralesional analgesic, via direct tumoral injection. The illumination process was done with different regimens for pain management (awake, sedation, or general anaesthesia). Initially we started with the awake mode, but this regimen was changed to intravenous sedation or general anaesthesia because of the difficulty in controlling local pain during treatment; patients were treated under intravenous sedation if they were medically unfit for general anaesthesia. Each patient received one round of photochemical internalisation; re-treatment was out of the scope of this trial. Any other clinically indicated investigations or interventions were implemented without delay and according to the patient's best interests.

In the immediate post-treatment phase, patients' analgesic requirements were satisfied through special pain protocols. Initially we started with the analgesic ladder principle, which failed to control the local pain. After discussion with the pain team specialists, the standard regimen became a fentanyl transdermal patch (12 mcg/h for 72 h) with morphine sulphate (immediate release) as needed for breakthrough pain. Dose-escalation of 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, or laryngeal malignancies) because airway 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 $TPCS_{2a}$ administration (day 0).

General medical assessment was done at selected follow-up visits as per protocol. Monitoring of vital signs, ECOG performance status, and adverse events took place at selected follow-up visits as per protocol. Radiographic assessments (lesion measurements) were done at day 0 (baseline), day 28, and 3 months. Standard laboratory monitoring (blood tests) consisted of full blood count (haemoglobin, platelets, and inflammatory markers), kidney function test (urea, creatinine, and electrolytes), liver function test (intrahepatic and extrahepatic enzymes), bone profile (calcium, phosphate, and related enzymes), and plasma glucose concentration. These assessments were done at day –14, baseline (day 0), day 4, day 14, day 28, and at 3 months.

Drug-related toxicity was defined and recorded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) version 3.0 (appendix p 1). Since major reactions in the illuminated area would mainly be caused by intentional destruction of tumour tissue, grade 1–3 toxicity was accepted inside the 5 cm zone of illumination (whereas only grade 1 toxicity was accepted outside the treated area). Adverse events were recorded and reported according to International Council for Harmonisation Good Clinical Practice guidelines. Pain was scored by the patient on a 10 cm visual analogue scale (VAS) on day 4 (just after light application) and day 5 (0 cm represented no pain and 10 cm most severe pain).

Blood and urine samples for pharmacokinetic analysis were taken on day 0 (before $TPCS_{2a}$ administration, and 30 min and 4 h after $TPCS_{2a}$ administration); on days 2, 4, 7, 14, and 28; and at the last visit (ie, at 3 months) (appendix p 2). Content of $TPCS_{2a}$ in plasma and urine was analysed by fluorescence spectroscopy.

Skin photosensitivity was assessed and recorded at specified intervals throughout the trial (days 0, 1, 3, 6, 14, and 28, and at 3 months). Skin photosensitivity tests were done with white light at two intensities: 500 lux (similar to bright indoor light) and 100 000 lux (similar to direct sunlight) for periods ranging from 30 s to 5 min (appendix p 2). Separate 0·8 cm² spots on the inside of the arm were exposed to light and patients were assessed at 1 h and 24 h after exposure. It was judged to be important to report any local skin changes, including erythema, oedema, blister formation, hypopigmentation, hyperpigmentation, scarring, atrophy, induration, and skin defects, because these could indicate phototoxicity. The scoring of skin photosensitivity was descriptive; no scale or grading system was used.

A single target area or lesion was identified in each participant on day –14 and treatment progress of that area was documented. All malignant lesions or areas were assessed and treated within each patient. Target lesion or area measurements by clinical examination (largest diameter), and ultrasonography when applicable, were recorded at days –14, 0, and 28, and at 3 months. Clinical photography was done on days 0, 7, 14, and 28, and at 3 months (appendix p 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 Tumors (RECIST) version 1.1 under the categories complete response, partial response, stable disease, or

progressive disease. Confirmed RECIST responses were those that had been confirmed at day 28 (appendix p 1). Surgical biopsies were done to assess the status of the treated margins with the sole aim of guiding any future treatment if necessary. We did not gather any biopsy data for the concentration of $TPCS_{2a}$ in the tumours on different days because this was not part of the clinical protocol. Patients were followed-up to assess survival at fixed intervals starting with day 28, month 3, every 3 months for 1 year, every 6 months for 2 years, and then annually.

Outcomes

The primary endpoints were safety and tolerability of $TPCS_{2a}$ in all treated cohorts. The primary endpoints were investigator assessed. Other co-primary endpoints were dose-limiting toxicity and maximum tolerated dose of $TPCS_{2a}$. When a patient reported any unacceptable toxicity, this was defined as dose-limiting toxicity recorded according to CTCAE (appendix p 1). The maximum tolerated dose is the dose at which 33% of patients within a cohort reported unacceptable toxicity.

The secondary endpoints were assessment of skin photosensitivity and the pharmacokinetics of $TPCS_{2a}$ from blood and urine samples. Antitumour activity was also documented as a co-secondary endpoint, and was assessed according to RECIST version 1.1; the tumour therapeutic depth represented the depth of therapeutic changes (depth of necrosis) achieved by the photochemical internalisation process in the target lesion for every patient and was confirmed clinically and radiologically, when indicated. When comparing therapeutic effect between dose cohorts, we use the term better therapeutic effect to mean an achievement of deeper tumour necrosis with minimal treatment-related adverse events. Depending on the target lesion site, the depth of effect (ie, the depth of tumour necrosis achieved by the intervention) was measured using a ruler or ultrasound, or both. Overall survival (defined as the length of time from the start of treatment until the death of the patient) was a post-hoc endpoint.

Statistical analysis

The aim of this study is to determine whether (or not) photochemical internalisation is safe and tolerable as an intervention. A statistical power calculation was done with the following parameters: desired power of 0·9, desired significance level α of 0·01, smallest clinically important difference resulting from photochemical internalisation treatment of 1 (an experimentally determined score), and standard deviation of the effect of photochemical internalisation of 1. The latter two variables were obtained from previous studies of photochemical internalisation.[4,13] The power calculation implied that a minimum of 18·3 patients were needed to observe a significant effect of photochemical internalisation.

The patients were defined as not assessable if there were insufficient data to enable their assessment for a specific category (ie, primary or secondary endpoint). This definition could include patients lost to follow-up, those who died during follow-up, suboptimally treated patients, and patients who had to leave the trial to receive further interventions.

The primary endpoints were analysed per protocol. The secondary endpoint of assessment of skin photosensitivity was assessed in all patients; pharmacokinetics of $TPCS_{2a}$ and antitumour activity were assessed in all patients apart from those in the dose de-escalation cohort. Pharmacokinetic analyses consisted of calculating elimination rates, half-lives, and area under the curve (AUC) for blood concentrations of $TPCS_{2a}$ using the non-compartmental method based on the log-trapezoidal rule. Safety and response data were summarised using descriptive statistics by dose. The safety population consisted of all patients, including those excluded from the trial for not reaching day 28. Descriptive statistical analysis comparing the response of the target lesion to treatment with adverse effects caused by photosensitivity was done with SPSS version 20.0.0. The 95% CI for the median overall survival follow-up data was calculated using the method of Brookmeyer and Crowley, based on inverting a sign test. We did not do any sensitivity analyses.

This study is registered with ClinicalTrials.gov, number NCT00993512.

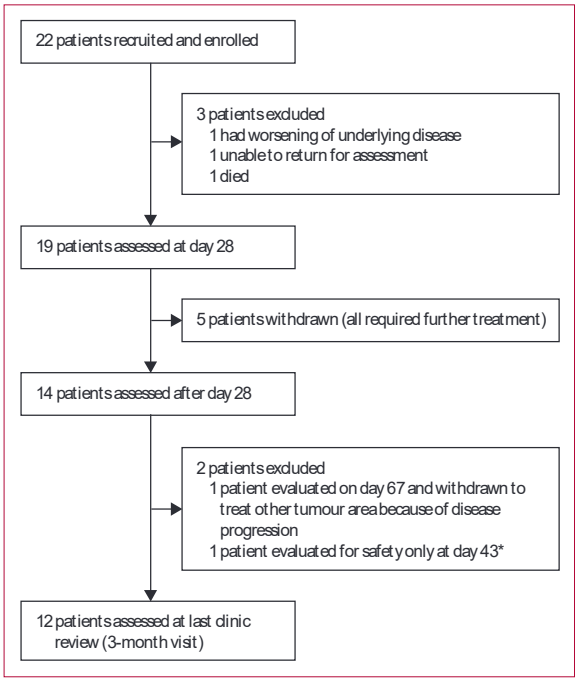

Figure 1: **Trial profile**
*This patient had to leave the trial to receive treatment for another tumour, which wasn't treated as part of the trial.

	Study population (n=22)
Sex	
Male	11 (50%)
Female	11 (50%)
Age (years)	60·0 (49·75–71·25; 34–82)
Ethnic origin	
White	20 (91%)
Asian	2 (9%)
Fitzpatrick skin type	
Type I	4 (18%)
Type II	7 (32%)
Type III	8 (36%)
Type IV	3 (14%)
ECOG performance status	
0	10 (45%)
1	9 (41%)
2	3 (14%)
Diagnosis	
Squamous cell carcinoma	16 (73%)
Sarcoma	1 (5%)
Ductal carcinoma	4 (18%)
Eccrine (adnexal) carcinoma	1 (5%)
Target lesion location	
Head and neck	17 (77%)
Torso, front	3 (14%)
Torso, back	1 (5%)
Arm	1 (5%)
Target lesion longest diameter (mm)	30·5 (27·25–46·25; 15–120)
Target lesion depth (mm)	16·5 (8·0–23·5; 2–38)

Data are n (%) or median (IQR; range). EGOG=Eastern Cooperative Oncology Group.

Table 1: **Baseline characteristics**

Role of the funding source

The funder of the study was involved in study design and writing of the report, but had no role in data collection, analysis, or interpretation. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Between Oct 3, 2009, and Jan 14, 2014, 22 patients were enrolled into the trial (figure 1). All enrolled patients were assessable at the time of joining the trial. Table 1 shows the baseline characteristics of enrolled participants. Most patients had squamous cell carcinoma of the head and neck, but some had other advanced or recurrent malignancies of the head and neck, torso, and upper limbs including sarcoma, eccrine (adnexal) carcinoma, and chemoresistant ductal breast carcinoma (table 1). All patients had previously received at least one surgical treatment for the same treated area along with chemoradiation.

Four patients were enrolled in the $TPCS_{2a}$ 0·25 mg/kg cohort (starting dose). The dose escalation proceeded according to a modification of Simon's accelerated titration design[19] in which the number of patients recruited depended on the dose-limiting toxicity recorded. Thus, three patients were included in each of the dose-escalation cohorts (0·5, 1·0, and 1·5 mg/kg). An optimal dose cohort (0·5 mg/kg) was selected on the basis of the dose-limiting toxicity and maximum tolerated dose information, which were actively examined during the trial, as well as the therapeutic depth of effect (see appendix p 3 for characteristics of the individual dose cohorts). Six more patients were recruited for this cohort with the aim to confirm findings (thus, a total of nine patients were in the 0·5 mg/kg cohort). Three patients were included in the dose de-escalation cohort (0·125 mg/kg).

19 of 22 treated patients reached the 4-week follow-up when dose-limiting toxicity and maximum tolerated dose were assessed; all safety data were also acquired. One patient (in the 1·0 mg/kg cohort) died in the first 4 weeks following treatment as a result of stroke. Two other patients (both in the 0·5 mg/kg cohort) left the trial before day 28: one to undergo further treatment and the other because he was unable to attend for further assessments; however, both patients were assessed on day 28 before leaving the trial. Between day 28 and the final clinic review at 3 months, seven patients left the trial to receive further treatment. 12 of 22 patients completed the trial to the 3-month follow-up visit (figure 1).

Administration of $TPCS_{2a}$ was found to be safe and tolerable by all patients. No clinically meaningful changes in vital signs were recorded compared with baseline; furthermore, there were no consistent patterns of change over time in mean haematological and blood biochemistry profiles (data not shown). $TPCS_{2a}$ and the photochemical internalisation treatment did not cause any negative direct effects on any of the monitored body organs (data not shown). 12 (63%) of 19 patients had no change in ECOG performance status between the pre-study visit and 3 months, two (11%) patients (both in the 1·5 mg/kg cohort) had an improvement, three (16%) worsened (one in the 0·5 mg/kg group and two in the 1·0 mg/kg group), and two patients had no baseline data (appendix p 4).

No adverse events occurred within the first 96 h of $TPCS_{2a}$ injection before the administration of bleomycin and the initiation of the photochemical internalisation treatment. Nine adverse events were reported in the lowest dose (0·125 mg/kg) cohort, four of which were related to photochemical internalisation (table 2). The number of adverse events reported in each of the dose cohorts was six in the 0·25 mg/kg cohort (five related to photochemical internalisation), 47 in the 0·5 mg/kg cohort (ten related to photochemical internalisation), seven in the 1·0 mg/kg cohort (one related to

photochemical internalisation), and 35 in the 1·5 mg/kg cohort (19 related to photochemical internalisation). In general, mild adverse events were reported in 15 patients, moderate adverse events in ten patients, and total severe adverse events in 14 patients (ie, any grade ≥3 adverse event: unexpected localised pain, severe localised infection, severe photosensitivity skin reaction [pruritus], respiratory failure, and death); some patients had more than one severe adverse event.

Adverse events related to photochemical internalisation were either local or systemic; the only one that was unexpected was localised high pain level (grade 3; reported in nine patients in total; table 2). Our clinical observation data suggested that pain was reported a few minutes after initiating the illumination procedure and escalated to maximum levels, then started to decline 1–2 h later and returned to reasonable (clinically expected) levels at 5–7 h. Mean pain scoring was highest in the first cohort (0·25 mg/kg) immediately after light delivery (8·08 cm [SD 0·04] on the VAS), because patients in this cohort were treated with locoregional anaesthesia only, and dropped to a mean of 1·45 cm [SD 0·8] after 24 h. All subsequent patients received general anaesthesia or sedation (along with locoregional anaesthesia), resulting in substantially better pain control as reflected by lower mean VAS scores (pain after illumination *vs* pain 24 h after: 4·28 cm [SD 1·1] *vs* 2·62 cm [1·3] in the 0·5 mg/kg cohort; 1·70 cm [0·8] *vs* 0·00 cm [0·0] in the 1·0 mg/kg cohort; 5·05 cm [0·2] *vs* 1·72 cm [0·5] in the 1·5 mg/kg cohort; and 7·67 cm [0·3] *vs* 2·47 cm [0·2] in the 0·125 mg/kg cohort). In all groups, the VAS score was substantially reduced 24 h after light delivery in all the dose cohorts, with no obvious dose relation.

Cancer-related adverse events were all expected (appendix p 5). Dysphagia (resulting from tumour growth) was reported in three patients (two in the 0·5 mg/kg cohort and one in the 1·0 mg/kg cohort), and was unrelated to the photochemical internalisation treatment field. Two patients (one in the 0·5 mg/kg cohort and one in the 1·0 mg/kg cohort) had locoregional haemorrhage caused by tumour invading the blood vessel walls and one of these patients also developed a fistula, which formed in the local tumour area. Respiratory failure (grade 4) was reported in two patients (one in the 0·5 mg/kg cohort and one in the 1·0 mg/kg cohort; see appendix p 6 for general medical and mental health-related adverse events). Most adverse events related to photochemical internalisation (reported once or more) were seen in the 1·5 mg/kg cohort (table 2). No patient required dose reductions and no patient discontinued the trial because of drug-related toxicity. No deaths related to photochemical internalisation occurred.

No adverse events corresponding to the definition of dose-limiting toxicity were recorded in the first two cohorts (0·25 mg/kg and 0·5 mg/kg). One dose-limiting toxicity

	Adverse events grade 1–2	Adverse events grade 3
0·125 mg/kg cohort (n=3)		
Localised erythema	2 (67%)	0
Localised swelling	1 (33%)	0
Nausea or vomiting	1 (33%)	0
Photosensitivity skin reaction, simple	0	0
Localised infection	0	0
Localised sensory disturbance	0	0
Photosensitivity skin reaction, pruritus	0	0
Unexpected localised pain	0	0
0·25 mg/kg cohort (n=4)		
Localised erythema	1 (25%)	0
Localised swelling	1 (25%)	0
Nausea or vomiting	0	0
Photosensitivity skin reaction, simple	0	0
Localised infection	0	0
Localised sensory disturbance	0	0
Photosensitivity skin reaction, pruritus	0	0
Unexpected localised pain	0	3 (75%)
0·5 mg/kg cohort (n=9)		
Localised erythema	1 (11%)	0
Localised swelling	1 (11%)	0
Nausea or vomiting	2 (22%)	0
Photosensitivity skin reaction, simple	0	0
Localised infection	2 (22%)	0
Localised sensory disturbance	0	0
Photosensitivity skin reaction, pruritus	0	0
Unexpected localised pain	0	4 (44%)
1·0 mg/kg cohort (n=3)		
Localised erythema	0	0
Localised swelling	0	0
Nausea or vomiting	0	0
Photosensitivity skin reaction, simple	0	0
Localised infection	0	0
Localised sensory disturbance	0	0
Photosensitivity skin reaction, pruritus	1 (33%)	0
Unexpected localised pain	0	0
1·5 mg/kg cohort (n=3)		
Localised erythema	3 (100%)	0
Localised swelling	3 (100%)	0
Nausea or vomiting	3 (100%)	0
Photosensitivity skin reaction, simple	3 (100%)	0
Localised infection	1 (33%)	1 (33%)
Localised sensory disturbance	1 (33%)	0
Photosensitivity skin reaction, pruritus	1 (33%)	1 (33%)
Unexpected localised pain	0	2 (67%)

Data are number of patients with at least one event (% of cohort population). All grade 1–2 adverse events occurring in 10% or more of patients and all grade 3 events occurring in the safety population are shown. No treatment-related grade 4 adverse events or treatment-related deaths occurred in this trial. $TPCS_{2a}$=disulfonated tetraphenyl chlorin.

Table 2: **Adverse events related to photochemical internalisation, by $TPCS_{2a}$ dose cohort**

(grade 2 photosensitivity skin reaction [pruritus]) was seen in the 1·0 mg/kg cohort and the next dose level chosen was therefore 1·5 mg/kg. Two of the three patients in the 1·5 mg/kg cohort reported dose-limiting toxicities. 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 protocol recommendations. The dose-limiting toxicity in the other patient was a grade 3 wound infection. These dose-limiting toxicities led to the conclusion that the maximum tolerated dose for $TPCS_{2a}$-mediated photochemical internalisation of bleomycin had been exceeded, and the maximum tolerated dose of $TPCS_{2a}$ was therefore agreed to be 1·0 mg/kg.

The mean plasma concentration of $TPCS_{2a}$ after administration in the four higher dose cohorts is shown in figure 2. We excluded the three patients in the dose de-escalation (0·125 mg/kg) cohort from the pharmacokinetic analysis because they were considered to have had a suboptimal intervention, and two of these three patients were re-treated, outside of the trial, with photochemical internalisation (0·5 mg/kg $TPCS_{2a}$) during the pharmacokinetic assessment period. The highest mean $TPCS_{2a}$ concentration was recorded at the sample timepoint 30 min (0·02 days) after $TPCS_{2a}$ administration. For the 0·25, 0·5, and 1·0 mg/kg doses, there was a near proportional relation between dose and mean maximum concentration, whereas the increase in maximal concentration from the 1·0 mg/kg to the 1·5 mg/kg dose seemed to be less than proportional. After a rapid first phase of elimination, concentrations of $TPCS_{2a}$ decreased 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 concentration of $TPCS_{2a}$ was higher than on day 4 (figure 2; appendix p 7).

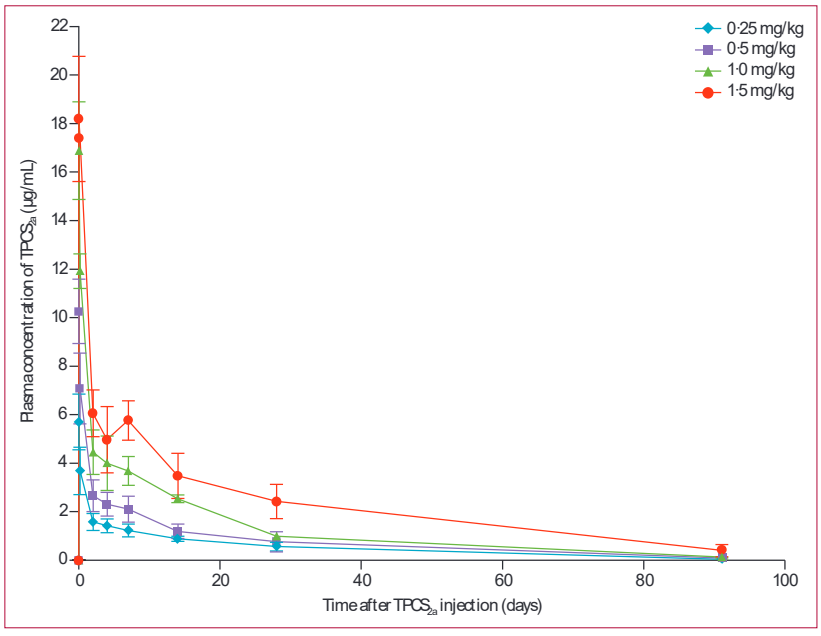

Figure 2: **Mean plasma concentration of disulfonated tetraphenyl chlorin ($TPCS_{2a}$)**
The concentration of $TPCS_{2a}$ in plasma samples from patients in the dose cohorts 0·25–1·5 mg/kg was analysed by fluorescence spectroscopy. Error bars show SD.

The pharmacokinetic behaviour of $TPCS_{2a}$ was further analysed by a non-compartmental approach based on the last four measurements for each patient. There was no notable difference between doses in the mean elimination rates, nor in the mean elimination half-life (appendix p 7). As expected, the mean values of $AUC_{0-\infty}$ increased with increasing dose (appendix p 7). $TPCS_{2a}$ was still detectable in blood 90 days after administration of all evaluable doses. No $TPCS_{2a}$ was detectable in urine in the first 14 patients; therefore, we decided not to analyse urine samples from the remaining patients.

No photosensitivity reactions were reported in any patient to exposures of 500 lux (bright indoor light). At 100 000 lux (equivalent to direct sunlight), photosensitivity was detected in at least one patient in all cohorts apart from the 0·125 mg/kg cohort (appendix p 8). All except one of the reactions were reported between day 3 and the last visit. All reactions were mild, apart from in one patient (1·5 mg/kg cohort) who had moderate (grade 3) oedema and erythema. Most of the reactions resolved within 24 h, but two patients (1·0 mg/kg and 1·5 mg/kg cohorts) required regular re-dressings for 1 week. One patient from the 1·5 mg/kg cohort required oral antibiotics for 5 days and regular re-dressings for 2 weeks. There seemed to be a correlation between both the frequency and duration of observed photosensitivity reactions and $TPCS_{2a}$ dose on the basis of qualitative assessment (data not shown). This concept can be exemplified by the finding of skin photosensitivity at day 90 in the 1·5 mg/kg cohort and the absence of photosensitivity reactions beyond day 14 in the 0·25 mg/kg cohort.

12 of 22 patients reached the final visit at 3 months. Of the ten patients who did not reach the final visit, three didn't reach day 28 (one died) and seven had to leave between day 28 and the final visit to receive further treatment (this includes the three patients from the suboptimally treated cohort who were also excluded from the tumour analysis). RECIST assessments for target lesions were available for 16 (84%) of 19 patients at day 28. The starting dose of $TPCS_{2a}$ (0·25 mg/kg) was not predicted to trigger a photochemical internalisation response; however, a localised synergistic effect with photoactivation was seen. Overall, across all dose cohorts, at day 28, complete response was achieved in 11 (58%) of 19 patients, partial response in two (11%) patients, stable disease in two (11%) patients, progressive disease in one (1%) patient, and data were recorded as missing for three (15%) patients (table 3). Furthermore, the outcome at the final clinic review at 3 months was complete response in six (50%) of 12 patients, partial response in

two (17%) patients, stable disease in two (17%) patients, and progressive disease in two (17%) patients (table 3). The effects of treatment on target lesion response were not confined to squamous cell carcinomas but were also seen in other tumour types such as sarcoma and chemoresistant ductal breast carcinoma, which have traditionally been very resistant to most treatment modalities (appendix p 9). Data for response by tumour type and dose are presented in the appendix, p 10.

At 28 days, target lesions had completely resolved in all four patients in the 0·25 mg/kg cohort. In the 0·5 mg/kg cohort, four (44%) of seven assessed patients had a complete response (table 3). Because the original dose escalation had shown good efficacy even with the lowest dose of 0·25 mg/kg, we decided to assess the clinical response at a lower dose of 0·125 mg/kg (dose de-escalation). However, the clinical response in this group was inferior to that seen in the higher dose groups; therefore, we did not include the dose de-escalation group in our RECIST assessments.

Five patients died during the trial, all from complications of the disease at distant organs. Two patients with pre-existing lung metastasis died of pulmonary haemorrhage, one patient with poorly controlled asthma and a long-standing history of opiate consumption died from multiorgan failure, and two patients had a cardiorespiratory arrest (one patient with a history of ischaemic heart disease had a heart attack just after having a stroke, and the other patient had lung metastasis, which progressed to the same outcome). There was no evidence of lung toxicity from bleomycin.

In patients with cutaneous malignancies, the malignant area turned necrotic after illumination, whereas the surrounding normal skin, although illuminated, remained intact. Similarly, when a subcutaneous malignancy was illuminated (in a patient in the 0·5 mg/kg cohort), the cancerous lesion became necrotic with no damage to the illuminated healthy overlying skin.

Promising antitumour activity was seen with $TPCS_{2a}$ doses of 0·25 mg/kg and higher, but a greater depth of therapeutic change (ie, depth of necrosis achieved by the photochemical internalisation) was seen in the 0·5 mg/kg cohort, which was selected for expansion. Although the maximum tolerated dose in the study was 1·0 mg/kg, in the dose-escalation part of the study, this dose did not give a better therapeutic effect (ie, a deeper tumour necrosis effect with minimal treatment-related adverse events; table 2) than the dose below it (0·5 mg/kg), which is why we chose 0·5 mg/kg for dose expansion. This choice was based on depth of effect at the whole tumour site and not just the target lesion. Data for the whole tumour site showed that the mean depth of effect was higher for 0·5 mg/kg than for 0·25 mg/kg (22·4 mm [SD 10·0] *vs* 19·3 mm [9·0]; appendix p 3). However, when the data for the patients in the 0·5 mg/kg cohort were analysed after the group had been expanded, the mean depth of effect in this dose was not found to be better than that in the 0·25 mg/kg cohort (17·2 mm [SD 12·6] *vs* 19·3 mm [9·0]; appendix p 3). In fact, on the basis of the target lesion data only, the 0·25 mg/kg cohort had a better overall effect (better tumour therapeutic depth and complete response outcome) than the 0·5 mg/kg cohort (mean depth of effect was 14·4 mm [SD 8·2] before expansion and 15·2 mm [6·3] after expansion in the 0·5 mg/kg cohort *vs* 17·5 mm [9·5] in the 0·25 mg/kg cohort), and fewer photosensitivity reactions than in the 0·5 mg/kg cohort, and therefore we recommend that 0·25 mg/kg should be the dose used in future trials. Mean target-lesion depth for complete responders at day 28 was 14·5 mm (SD 9·2), with the minimal depth being 4 mm and maximal being 25 mm. Mean target-lesion depth for complete responders at 3 months was 15 mm (SD 9·2), with the minimal depth being 4 mm and maximal being 25 mm (appendix p 11).

Median follow-up for all 22 patients was 11·7 months (IQR 4·8–24·7) and median follow-up for all patients (excluding the three suboptimally treated cohort 0·125 mg/kg) was 15·6 months (5–27·2). Median overall survival of all patients excluding the 0·125 mg/kg de-escalation cohort was 15·4 months (95% CI 5·9–24·9), and several patients with locally recurrent malignancies (without distant metastases) had remarkably long survival, with two patients alive more than 4 years after treatment, and an additional four patients living more than 2 years after treatment.

	0·25 mg/kg cohort	0·5 mg/kg cohort	1·0 mg/kg cohort	1·5 mg/kg cohort
Total number of patients*	4	9	3	3
Response at day 28				
Complete response	4 (100%)	4 (44%)	2 (67%)	1 (33%)
Partial response	0	0	0	2 (67%)
Stable disease	0	2 (22%)	0	0
Progressive disease	0	1 (11%)	0	0
Missing†	0	2 (22%)	1 (33%)	0
Number of patients assessed at 3 months	3	6	1	2
Complete response	3 (100%)	2 (33%)	0	1 (50%)
Partial response	0	1 (17%)	0	1 (50%)
Stable disease	0	2 (33%)	0	0
Progressive disease	0	1 (17%)	1 (100%)	0

The responses were assessed according to Response Evaluation Criteria in Solid Tumors (RECIST). Three patients did not reach day 28; an additional seven patients did not reach the last visit (see figure 1). Of the 11 patients who had complete response at day 28, six continued to have complete response at the last visit, two patients (with stable disease at day 28) had to leave the trial after day 28 to receive further treatment for systemic progressive disease not related to the treated site, one was assessed and had partial response outcome at the last visit, and two were assessed and had progressive disease at the last visit. *Not including three patients in the 0·125 mg/kg cohort; these patients were excluded because they were deemed to have had a suboptimal intervention, and two were re-treated during the pharmacokinetic analysis. †Excluded from the analysis because they did not reach day 28.

Table 3: **Investigator-assessed target lesion response**

Discussion

Our results show that the new photosensitiser $TPCS_{2a}$, when used before photochemical internalisation of bleomycin, was safe and tolerated by all patients, and was an acceptable treatment option in a very complex population of patients with solid malignancies who had exhausted all conventional interventions for their disease before joining the trial. Dose-limiting toxicities were reported in two patients at a $TPCS_{2a}$ dose of 1·5 mg/kg; thus the maximum tolerated dose of $TPCS_{2a}$ was determined to be 1·0 mg/kg. Adverse events related to photochemical internalisation were either localised, resulting from the local inflammatory process, or systemic, mostly as a result of the skin-photosensitising effect of $TPCS_{2a}$. No treatment-related deaths were recorded. One of the most striking findings from this study is the dramatic tumour responses reported. The starting dose of $TPCS_{2a}$ for the study was set at a level not expected to trigger a treatment response; however, there appeared to be a localised synergistic effect with photoactivation. This effect was not only confined to squamous cell carcinomas, but also affected tumours such as sarcoma, which have traditionally been very resistant to most treatment modalities.

The unexpected high levels of pain reported by patients during illumination when done under local anaesthesia were eliminated by the use of general anaesthesia or intravenous sedation. This discomfort was localised to the site of illumination. On the basis of clinical observations, the amount of pain seemed to be associated with the size of the surface area of tumour exposed to illumination. We postulate that the induction of acute necrosis with the release of intracellular degradation products might have stimulated small pain fibres either directly or through histamine, chemokine, and cytokine release.[20]

One of the dose-limiting toxic effects of $TPCS_{2a}$-mediated photochemical internalisation 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 such reactions. Controlled skin photosensitivity measurements suggested that skin photosensitivity was dose dependent, being substantially less frequent and less severe at the 0·25 mg/kg dose than at higher doses. Skin photosensitivity was reported for a notable period of time following $TPCS_{2a}$ administration, especially at higher doses, but was clinically manageable. This prolonged effect was also reflected in the pharmacokinetic measurements. There was no indication that the use of photochemical internalisation increased the reported skin toxicities of bleomycin,[21] which accords with findings from animal models in which administration of bleomycin did not increase skin photosensitivity over that reported with $TPCS_{2a}$ and illumination alone.[7] Our data suggest that direct sun exposure should be avoided for 3 months after $TPCS_{2a}$ administration. However, exposure to normal daylight (not direct sunlight) is possible after 2–3 weeks. Gradual sun exposure is recommended at an incremental rate of 100 lux per day.

A notable finding from our trial was that $TPCS_{2a}$-mediated photochemical internalisation of bleomycin induced strong tumour responses, even in this heavily pretreated population with advanced and recurrent malignancies. Although the maximum tolerated dose of $TPCS_{2a}$ was established to be 1·0 mg/kg, robust antitumour effects were seen at all doses from the starting dose of 0·25 mg/kg. An inferior response was noted in the de-escalation cohort of 0·125 mg/kg, but there was no clear dose response at doses of 0·25 mg/kg or higher. This interpretation is based on a small sample size; the cohort in which all patients achieved a complete response had only four patients (0·25 mg/kg), whereas the cohort in which a smaller proportion of patients achieved a complete response (0·5 mg/kg) had nine patients. The preliminary results on tumour response from our study suggest that a photochemical dose sufficient to produce a complete tumour response can be used without causing severe damage to surrounding normal tissue within the illuminated field.

Our findings suggest that photochemical internalisation has good potential for high tumour selectivity when treating cutaneous and subcutaneous malignancies. This suggestion of tumour selectivity was somewhat unexpected, since in mouse xenograft studies the distribution of $TPCS_{2a}$ has not been shown to discriminate well between tumour tissue and adjacent healthy skin.[7] However, in a head and neck cancer model in which tumour cells were grown in the cheek pouch of hamsters, some tumour selectivity of $TPCS_{2a}$ accumulation has been reported,[21] and major selectivity (5–7 times) between tumour tissue and underlying muscle tissue has been reported in mice.[7]

Bleomycin itself is not very tumour selective;[22] however, it is more toxic to highly proliferating cells than to highly proliferating tumour cells and thus the cellular uptake or the biological effect of bleomycin might be higher in cancer cells than in surrounding normal cells in the skin.[23,24] We would not expect the acute dose-limiting side-effects of bleomycin, including myelosuppression, to be increased by a locally directed therapy such as photochemical internalisation, and our results support this theory. Additionally, cumulative pulmonary toxicity reported with bleomycin[25] is mitigated by the fact that photochemical internalisation treatment is only a single administration.

In preclinical studies, photochemical internalisation enhanced the effect of endocytosed molecules even when the target cells were exposed to subtoxic illumination doses,[15,26,27] which suggests that photochemical internalisation might be able to produce deeper effects than those achievable with pure photodynamic therapy.[27,28] However, it was rather surprising that in our study complete tumour responses were seen in tumours with a

depth of up to 38 mm. The induction of an immunological response, which has been described for photochemical treatments, could also explain the selectivity of the treatment.[29,30] In addition to the possible immune-stimulating induction of necrosis, inflammation, and cytokine production,[31] photochemical internalisation has recently been reported to be able to enhance MHC class I antigen presentation—an important factor in the generation of an effective immune response to tumours.[31,32] Both photodynamic therapy and photochemical internalisation have shown notable effects on tumour vasculature,[27] which might justify the therapeutic effects at these deep levels. Furthermore, preclinical studies show that photochemical internalisation of bleomycin has the potential to induce antitumour immunity in human beings (Norum OJ, Fremstedal ASV, Weyergand A, Golab J, Berg K, Oslo University Hospital, personal communication).

This phase 1 study did not specifically include observation of metastatic non-treated tumours in the protocol. However, both innate and adaptive immune responses are generally reported after photodynamic therapy.[29] However, antitumour immunity might not be efficient for several reasons, such as the absence of HLA class I expression on the tumour cells or regulatory T-cell immune suppression.[33] Thus, photochemical internalisation might activate the innate immune system without triggering an adaptive response. Natural killer cells have also been shown to act more efficiently on MHC class I-negative tumours.[34] We therefore envision that a strong innate immune response might induce tumour cell death in areas of the tumour with suboptimal treatment responses such as in deep tissue layers.

One of the limitations of this study was the small sample size, which led to an even smaller sample when the participants were divided into the different dose cohorts. Furthermore, several patients had to leave the trial before the 28-day and final assessments to receive treatment for non-treated areas or distant disease. The extensive disease in many of the patients made identification of an easy-to-assess target lesion challenging. Furthermore, the numerous collections of blood and urine samples for pharmacokinetic assessment and the follow-up visits posed some difficulties to our population of patients with advanced disease. Additionally, recruitment of patients for this phase 1 study was challenging because the trial involved a new drug, which had not been tested in human beings before.

An interesting aspect of photochemical internalisation technology is that $TPCS_{2a}$ and similar molecules, by contrast with many other photosensitisers, are not affected by many common drug resistance mechanisms.[35,36] 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.[36] Thus, photochemical internalisation has the potential to treat tumours with acquired drug resistance[37] and perhaps even inherently chemoresistant cancer stem cells.

Photochemical internalisation technology has potential beyond its use with cytotoxic drugs, such as use with macromolecular agents. Many macromolecules are totally dependent on endosomal release to reach intracellular targets and therefore have the potential to greatly enhance activity when combined with photochemical internalisation. A particularly interesting option is to combine photochemical internalisation with antibody-based molecules, such as immunotoxins or antibody–drug conjugates with intracellular targets. In-vitro and in-vivo studies have shown that photochemical internalisation can enhance the effect of immunotoxins directed to several different relevant cancer surface markers such as EGFR[37,38] and others.[39,40]

In theory, photochemical internalisation could be used to treat all solid tumours. This treatment could also be highly suitable for early-stage cancers, as a neoadjuvant to standard treatment procedures and for the treatment of innate or acquired treatment-resistant tumours. The only difference from standard radio-oncological therapy is the use of a light source, and with a less challenging group of patients than that included in this phase 1 study, we envision that photochemical internalisation could be done in most surgical departments and even outside the operating theatre. The only limitation is the need for local or general anaesthesia, or both, because of the observed high level of pain during the light exposure phase, although pain after treatment can be managed easily.

A multicentre phase 2 trial (NCT01606566) in patients with head and neck cancer was started in May, 2012, at several centres in Germany and France, with the 0·25 mg/kg dose of $TPCS_{2a}$. This study included patients receiving superficial illumination (as in our phase 1 study), and those with larger tumours receiving interstitial illumination with multiple optical fibres inserted into the tumour. The responses seen in the patients assigned to superficial illumination were similar to those described here. However, for the interstitially treated tumours, the placement of the fibres and dosing of the illumination was challenging. The study was terminated before being completed, mainly because of strategic commercial considerations from the study sponsor. However, a phase 1–2 study assessing photochemical internalisation for enhancing the effect of gemcitabine in patients with cholangiocarcinoma is in progress, with promising results in the dose-escalation part of the study (Berg K, Høgset A, unpublished).

In summary, the promising results of $TPCS_{2a}$-mediated photochemical internalisation of the chemotherapeutic agent bleomycin in this heavily pretreated population suggest that this treatment could have an important role in interventional oncology. The uniform effect of photochemical internalisation in causing tumour death in a range of very aggressive cutaneous and subcutaneous

malignancies including squamous cell carcinoma, sarcoma, eccrine (adnexal) carcinoma, and chemo-resistant ductal carcinoma, with preservation of adjacent, non-malignant tissues, was encouraging. Our study paves the way for further clinical development of $TPCS_{2a}$-mediated photochemical internalisation of bleomycin, and for clinical testing of the technology with other drug molecules on different tumour types.

Contributors

AAS, WJ, KB, CAM, ZH, DC, MF, and CH contributed to study conception, study design, and data collection, and participated in the literature search, and preparation and review of the report. AH and RH contributed to study conception, study design, and data analysis, and participated in the literature search, and preparation and review of the report. CS contributed to data collection, and participated in the literature search, and preparation and review of the report. All authors read and approved the final version of the report.

Declaration of interests

AH works as Chief Scientific Officer for PCI Biotech AS. AH reports grants from Norwegian Research Council, during the conduct of the study. AH has a patent PCT/GB00/00903 WO00/54802 issued, a patent PCT/GB01/05299 WO02/44396 issued, a patent PCT/GB2009/001618 WO2010/01102 issued, a patent PCT/EP2014/068313 pending, a patent PCT/EP2014/068236 pending, and a patent PCT/GB2010/001547 issued. All other authors declare no competing interests.

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