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Clinical Trial Results

Photochemical Internalization of Gemcitabine Is Safe and Effective in Locally Advanced Inoperable Cholangiocarcinoma

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Abstract

Background: Photochemical internalization (PCI) is a novel technology for light-induced enhancement of the local therapeutic effect of cancer drugs, utilizing a specially designed photosensitizing molecule (fimaporfin). The photosensitizing molecules are trapped in endosomes along with macromolecules or drugs. Photoactivation of fimaporfin disrupts the endosomal membranes so that drug molecules are released from endosomes inside cells and can reach their therapeutic target in the cell cytosol or nucleus. Compared with photodynamic therapy, the main cytotoxic effect with PCI is disruption of the endosomal membrane resulting in delivery of chemotherapy drug, and not to the photochemical reactions per se. In this study we investigated the effect of PCI with gemcitabine in patients with inoperable perihilar cholangiocarcinoma (CCA).

Methods: The in vitro cytotoxic effect of PCI with gemcitabine was studied on two CCA-derived cell lines. In a fimaporfin dose-escalation phase I clinical study, we administered PCI with gemcitabine in patients with perihilar CCA (n = 16) to establish a safe and tolerable fimaporfin dose and to get early signals of efficacy. The patients enrolled in the study had tumors in which the whole length of the tumor could be illuminated from the inside of the bile duct, using an optical fiber inserted via an endoscope (Fig. 1). Fimaporfin was administered intravenously at day 0; gemcitabine (i.v.) and intraluminal biliary endoscopic laser light application on day 4; followed by standard gemcitabine/cisplatin chemotherapy.

Results: Preclinical experiments showed that PCI enhanced the effect of gemcitabine. In patients with CCA, PCI with gemcitabine was well tolerated with no dose-limiting toxicities, and no unexpected safety signals. Disease control was achieved in 10 of 11 evaluable patients, with a clearly superior effect in the two highest dose groups. The objective response rate (ORR) was 42%, including two complete responses, while ORR at the highest dose was 60%. Progression-free survival at 6 months was 75%, and median overall survival (mOS) was 15.4 months, with 22.8 months at the highest fimaporfin dose.

Conclusion: Photochemical internalization with gemcitabine was found to be safe and resulted in encouraging response and survival rates in patients with unresectable perihilar CCA.

Key words: cholangiocarcinoma; fimaporfin; photochemical internalization; gemcitabine; endoscopic retrograde cholangiopancreatography.

Lessons Learned

- This open-label multicenter phase I study in patients with inoperable perihilar cholangiocarcinoma showed that photochemical internalization (PCI) combined with gemcitabine was safe.
- Encouraging clinical responses and survival rates were observed using PCI followed by standard of care gemcitabine/cisplatin.
- Together with an earlier study in head and neck cancer patients, this work indicates fimaporfin-PCI is feasible, safe, and might enhance the cytotoxic effect of chemotherapy.

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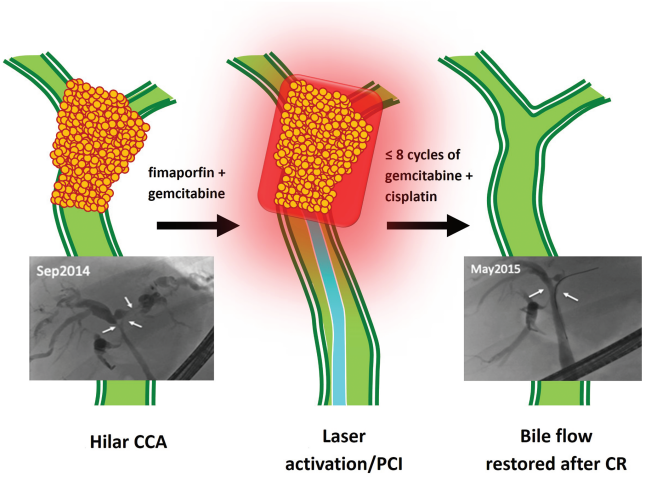

Figure 1. Treatment overview. An overview of the PCI treatment with an example of a radiological response in one of the study patients. The stenosis almost completely resolved, and the patient had PFS of 13 months and OS of 24 months.

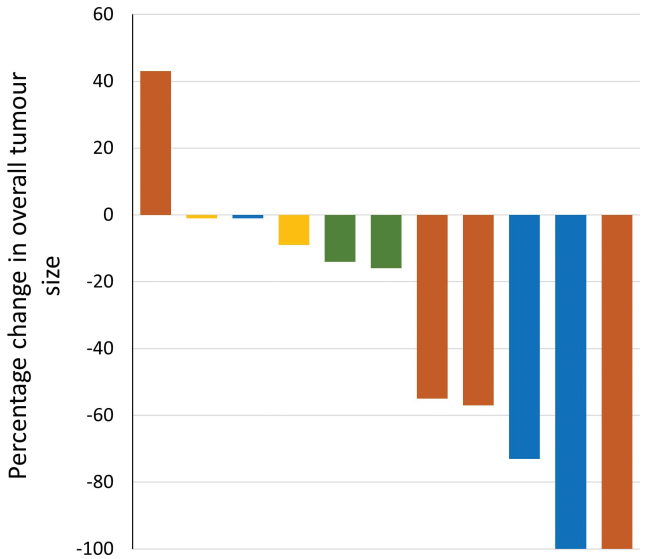

Figure 2. Effect of PCI treatment—best overall response. (**A**) Waterfall plot showing the percentage maximum reduction in target tumor size (sum of largest diameters) from baseline in all radiologically evaluable patients. Bar colors are: yellow: cohort 1; green: cohort 2; blue: cohort 3; brown: cohort 4.

Discussion

In this phase I fimaporfin dose-escalation study in patients with inoperable perihilar CCA, we demonstrated that PCI with gemcitabine could be safely administered preceding gem/cis chemotherapy. This combined treatment showed encouraging efficacy, and no unexpected safety signals were observed (Fig. 1). The most significant adverse event was cholangitis observed in 56% of patients. However, cholangitis is frequent in patients with CCA treated by biliary drainage only, and a similar frequency was observed in patients receiving standard treatment. Skin photosensitivity was observed in a substantial proportion (75%) of patients. As in the first-in-man PCI study in head and neck cancer patients, photosensitivity reactions were generally mild, with only two events of moderate blistering. Most photosensitivity reactions occurred within 30 days of fimaporfin administration, with very few seen after day 45.

Early signs of efficacy were promising, with a mOS of 15.4 months (n = 16), and 22.8 months at the highest fimaporfin dose explored (n = 6). In comparison, mOS in the ABC-02 trial establishing gem/cis as a standard-of-care therapy was 11.7 months. Photochemical internalization treatment led to shrinkage of almost all target lesions (Fig. 2), and 2 of 11 evaluable patients achived a CR, which compares favorably to the single CR observed in the phase III ABC-02 trial.

The PCI treatment regimen fits well into current treatment regimens for CCA, adding only minimal time and complexity to endoscopy drainage. An ongoing, global pivotal phase II study (ClinicalTrials.gov ID: NCT01900158) is evaluating PCI with gemcitabine in combination with gem/cis versus gem/cis alone.

Author disclosures and references available online.

Trial Information	
Disease	Biliary tract: gallbladder cancer and chloangiocarcinoma
Stage of disease/treatment	Metastatic/advanced
Prior therapy	None
Type of study	Phase I, 3+3
Primary endpoints	Toxicity, safety
Secondary endpoints	Recommended phase II dose, pharmacodynamics
Investigator's Assessment	Active and should be pursued further

Additional Details of Endpoints or Study Design

Clinical Study Design and Participants

The study was an open-label multicenter phase I study to assess the safety, tolerability, and efficacy of fimaporfin-induced PCI with gemcitabine, followed by systemic gem/cis chemotherapy in chemotherapy naïve patients with inoperable, advanced perihilar cholangiocarcinoma (CCA; Clinical trial number EudraCT No 2012-002888-10. Protocol registered in ClinicalTrials.gov 15 May 2013). Ethics approval was obtained from National Research Ethics Service Committee North West—Liverpool East, Manchester, UK (REC Ref: 12/NW/0739) and Ethik-Kommission—Landesärztekammer Rheinland-Pfalz, Mainz, Germany (Letter dated 05 April 2013), and all patients gave informed consent before taking part in this study.

Inclusion criteria included: (1) Histopathologically/cytologically verified adenocarcinoma consistent with locally advanced and inoperable cholangiocarcinoma; (2) nodal enlargement limited to the periportal, common hepatic artery and porta hepatis regions (N1 as per computed tomography [CT]/magnetic resonance imaging [MRI] assessment); (3) adequate biliary drainage with no evidence of active uncontrolled infection; (4) age ≥18 years; (5) Eastern Cooperative Oncology Group (ECOG) performance status ≤1; (6) life expectancy ≥12 weeks.

Patients with previous anti-cancer CCA treatment were excluded, as were patients with: (1) severe visceral disease other than CCA; (2) primary sclerosing cholangitis; (3) concomitant malignant disease (a second malignancy); and (4) inadequate bone marrow, liver, or renal function. Patients were also excluded if unable to undergo CT or MRI, or if they participated in any other interventional clinical trial.

Baseline tumor evaluations were performed up to one month prior to study registration. All patients had a follow-up visit at 30 days after the last administration of systemic chemotherapy, and survival status was documented until death.

All adverse events (AEs) and serious AEs (SAEs) were documented from the time of informed consent until 30 days after the last chemotherapy administration.

The primary objective of the study was to investigate the safety of the treatment, and to determine dose-limiting toxicities (DLTs). Key secondary endpoints were to determine: (1) progression-free survival (PFS) and (2) best overall response (BOR). Assessment of skin photosensitivity was an important exploratory endpoint. Sixteen patients were treated at eight centers in the UK and Germany.

Clinical Procedures

For the PCI treatment, patients in four cohorts (Table 1) received a single dose of fimaporfin on day 0, followed 4 days later (day 4) by a standard dose of gemcitabine infusion (1000 mg/m^2) and intraluminal laser light illumination. The patients enrolled in the study had tumors in which the whole length of the tumor could be illuminated from the inside of the bile duct, and ERCP was used to place an optical fiber across the tumor. Commencing 7-21 days after illumination, patients received up to eight cycles of standard systemic chemotherapy with cisplatin (25 mg/m^2) and gemcitabine (1000 mg/m^2), given on days 1 and 8 of each 21-day cycle.

The light source used was the CE marked PCI 652 nm laser (PCI Biotech AS). Intraluminal light application was performed 3 (±1) hours after the end of gemcitabine administration, using an optic fiber with a cylindrical light diffuser. A catheter was advanced into the stenosis and the fiber was inserted into the catheter. An irradiance of 100 mW/cm was employed in all cohorts giving illumination times of 150 s (15 J/cm dose) or 300 s (30 J/cm dose). Pain medication was administered as per local practice. To minimize the risk of photosensitivity reactions, light avoidance measures were initiated immediately after the fimaporfin injection for 14 days.

General medical examination and routine blood testing were performed at every patient visit. Adverse events were recorded and reported according to International Council for Harmonization (ICH) Good Clinical Practice (GCP) guidelines. Dose-limiting toxicities were defined and AEs recorded according to the Common Terminology Criteria for Adverse Events v4.02 (CTCAE). Response and progression were evaluated using Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 41. Overall survival and PFS were calculated from the time of patient registration. For skin photosensitivity assessment patients were asked daily to complete a questionnaire documenting their daily exposure to light, and were asked about light exposure and photosensitivity reactions at study visits for 3 months after fimaporfin administration.

Drug Information	
Fimaporfin	
Generic/working name	Fimaporfin
Company name	PCI Biotech AS
Drug type	Porphyrin-based photosentitizing molecule

Fimaporfin	
Drug class	Photosensitizer
Dose	0.06 to 0.25 mg/kg
Route	i.v.
Schedule of administration	One injection of fimaporfin was administered 4 days before laser light (wavelength 652 nm) illumination of the bile duct tumor, using an optical fiber inserted via an endoscope
Gemcitabine	
Generic/working name	Gemcitabine
Drug type	Small molecule
Dose	1000 mg/m^2
Route	i.v.
Schedule of administration	Gemcitabine was administered 2-4 hours before laser light illumination

Dose Escalation Table

Dose level	Dose of drug: fimaporfin	Dose of drug: gemcitabine, mg/kg	Number enrolled
Cohort 1	0.06 mg/kg fimaporfin, 15 J/cm illumination	1000	3
Cohort 2	0.06 mg/kg fimaporfin, 30 J/cm illumination	1000	3
Cohort 3	0.12 mg/kg fimaporfin, 30 J/cm illumination	1000	4
Cohort 4	0.25 mg/kg fimaporfin, 30 J/cm illumination	1000	6

Patient Characteristics

Number of patients, male	13
Number of patients, female	3
Age	Median (range): 64.3 (48-79) years
Number of prior systemic therapies	None
Performance Status: ECOG	0—14 1—2 2—0 3—0 Unknown—0
Other	Of the 16 enrolled patients, 4 had nonmeasurable disease at baseline and were not included in the efficacy evaluation, and 3 patients left the trial before the 3 months evaluation, leaving 11 patients for efficacy evaluation at 3 months. Between 3 and 6 months, 2 patients were withdrawn. Eleven patients completed the trial, of whom 10 were evaluable for efficacy. Additional patient characteristics, including tumor stage, can be found in Tables 3 and 4
Cancer types or histologic subtypes	Peri-hilar cholangiocarcinoma, 16

Primary Assessment Method

Title	Efficacy (RECIST)
Number of patients screened	16
Number of patients enrolled	16
Number of patients evaluable for toxicity	16
Number of patients evaluated for efficacy	12
Evaluation method	RECIST 1.1
Response assessment CR	*n* = 2 (16.7%)
Response assessment PR	*n* = 3 (25%)
Response assessment SD	*n* = 7 (58%)

Outcome Notes

General medical examination and routine blood testing were performed at every patient visit. AEs were recorded and reported according to ICH GCP guidelines. DLT was defined and AEs recorded according to the Common Terminology Criteria for Adverse Events v4.02 (CTCAE). Response and progression were evaluated using Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 41. OS and PFS were calculated from the time of patient registration. For skin photosensitivity assessment patients were asked daily to complete a questionnaire documenting their daily exposure to light, and were asked about light exposure and

photosensitivity reactions at study visits for 3 months after fimaporfin administration.

Adverse Events

Adverse events observed in the study are shown in Table 1. No DLTs were observed.

Serious Adverse Events		
Name	**Grade**	**Attribution**
Cholangitis	2	Unrelated
Cholangitis	3	Unrelated
Cholangitis	3	Probable
Hepatobiliary disease	3	Probable
Lower respiratory tract infection	3	Unrelated
Clostridial infection	2	Unrelated
Abdominal pain	3	Unrelated
Gastrointestinal haemorrhage	3	Unrelated
Impaired gastric emptying	3	Unrelated
Nausea	3	Unrelated
Vomiting	3	Unrelated
Atrial flutter	3	Unrelated
Pulmonary embolism	3	Unrelated

Altogether, there were 29 SAEs in 13 of 16 patients; 2 of these SAEs were considered probably related to the PCI treatment. All SAEs were grade 2 or 3. The most frequent SAEs were related to cholangitis (17/29), but only one of these was considered as probably related to the PCI treatment. All cholangitis SAEs have not been entered individually, but of the 17 events 7 were grade 2 and 10 were grade 3 (Table 1).

Assessment, Analysis, and Discussion	
Completion	Study completed
Investigator's Assessment	Active and should be pursued further

Photochemical internalization (PCI) is a technology for enhancing and directing the effect of drug molecules by illumination. Photochemical internalization works by releasing drug molecules from endosomes inside cells, so that the drug molecules can reach therapeutic target in the cell cytosol or nucleus. This effect is obtained by using an intravenously administered photosensitising compound, fimaporfin followed by illlumination of the lesion to be treated. Photoactivation of fimaporfin disrupts endosomal membranes thereby allowing release of the chemotherapeutic agent from the endosome. When compared with photodynamic therapy (PDT), the main cytotoxic effect with PCI is due to the delivered drug, and not to the photochemical reactions per se. This work demonstrates that fimaporfin-based PCI technology enhances the cytotoxic effect of gemcitabine in a light-dependent manner in CCA cell lines in vitro (Fig. 3); and a clinical phase I dose-escalation study in patients with inoperable perihilar CCA showed that a PCI treatment could safely be administered preceding the "standard-of-care" gem/cis chemotherapy; no DLTs or unexpected safety signals were observed. This treatment also showed encouraging efficacy data.

In the clinical study, the photochemical dose was escalated in four cohorts (Fig. 4), and very encouraging efficacy results were obtained with the 0.25 mg/kg fimaporfin/ 30 J/cm illumination combination employed in cohort 4. In an earlier phase I study of PCI with bleomycin and superficial illumination, the maximum tolerated fimaporfin dose was determined to 1 mg/kg, but the anti-tumor effect seemed just as good at doses of 0.25 and 0.5 mg/kg.[1] However, in a subsequent phase II trial in head and neck cancer 0.25 mg/kg fimaporfin combined with an intratumoral light dose of 60 J/cm was associated with serious local adverse reactions in the tumor-adjacent healthy tissue (unpublished data), suggesting that this combination represents an upper limit to light application inside a tumor. Since local tissue destruction in and around the bile duct may result in serious complications, we chose not to escalate the treatment doses above 0.25 mg/kg with 30 J/cm illumination, even though a formal DLT was not reached.

Since several studies with PDT indicate substantially enhanced therapeutic effects by repeating the PDT treatment,[2,3] the safety of repeated treatments with PCI was investigated in 6 additional patients. No additional safety signals were observed, indicating the safety of using repeated treatment in later studies.

Cholangitis (maximum grade 3) occurred in 56% of the patients (Table 1), which is not unexpected in patients with CCA requiring drainage. A similar cholangitis frequency has been observed in studies with CCA patients receiving PDT or stenting only[2,4] and for patients receiving PDT in combination with chemotherapy.[5] A recent small study comparing chemotherapy and stenting with and without temoporfin-PDT also showed equal rates of grades 3 and 4 cholangitis episodes (60% in both groups).[6] In the present study, there was no obvious correlation between PCI dose and frequency of cholangitis, nor did the occurrence of cholangitis events correlate in time with the PCI treatment (Fig. 5). This indicates that the cholangitis events in this study were not induced by the PCI treatment, but were related to the underlying disease or normal treatment procedures (e.g., stenting). Since possible serious local reactions like bile duct perforation were

not observed, the PCI treatment seems to have a good safety profile regarding local effects in the bile duct.

Other grades 3 to 4 AEs were mainly hematological or gastrointestinal; these are probably related to the gem/cis chemotherapy.[7,8]

Skin photosensitivity is a well-known side effect of photochemical cancer treatments and was observed in a substantial fraction (75%) of the patients in the present study. Most photosensitivity reactions occurred within 30 days of fimaporfin administration, with very few seen after day 45 (Fig. 5). As also observed in the first-in-man PCI study,[1] the photosensitivity reactions were generally mild and varied between patients, with two events of moderate blistering being the most severe reactions.

The efficacy results in this study compare favorably to those achieved with systemic therapies for inoperable CCA. The mOS for all 16 patients was 15.4 months, with an OS of 22.8 months for the 6 patients receiving the highest fimaporfin dose (Table 2). In comparison, the OS in the ABC-02 study establishing gem/cis as a standard-of-care therapy was 11.7 months.[7] The PFS at 6 months in this study was 75%, comparing favorably to the 59.3% observed for gem/cis treated patients in ABC-02. Since the present study only included patients with perihilar CCA and ABC-02 also included patients with other types of CCA, a direct comparison is difficult. However, a post hoc analysis of patients from the ABC-02 and -03 studies suggests that intrahepatic CCA treated with gem/cis chemotherapy has a mOS (15.2 months), longer than the other forms of CCA (extrahepatic, gallbladder, and ampulla vater) included in these studies.[9]

Given that many non-resectable CCA patients have severe symptoms, and often die, from the local disease, local treatments like PCI could prolong survival and enhance of quality of life. Thus, several studies have indicated a potential survival advantage for patients who have received PDT,[4,10,11] and some retrospective studies have indicated that patients receiving PDT combined with chemotherapy survived longer than patients receiving PDT alone.[5,12] However, there is also a recent publication describing PDT with stenting as inferior to stenting alone.[13] The reason for the discrepancy in results between this study and the other PDT studies is still unclear, and more studies are warranted to define the role of photochemical technologies in CCA treatment.

Most PDT studies have employed the photosensitiser Photofrin (activated at 630 nm), which has been reported to have tumoricidal effects up to 4 mm into the tumour.[14] With fimaporfin-PCI one would expect a significantly deeper antitumor effect, both because of better tissue light penetration at the 652 nm activation wavelength, and because the illumination dose for inducing endosomal drug release is significantly lower than the dose needed for killing tumor cells by PDT.[15]

The PCI treatment regimen fits well into current treatment regimens for CCA, adding only minimal time and complexity to the routine catheter procedure. The PCI treatment led to shrinkage of almost all target lesions (Figs. 2 and 6), and the two CRs (in 11 evaluable patients) observed in this study compare very favorably with the single CR observed among 204 gem/cis treated patients in the ABC-02 study.[7] The long survival times seen in some of the patients underscore the potential of the technology.[16] Thus, based on the promising safety and efficacy data observed in this study, a randomized pivotal phase II study in the same patient population is on-going, to include 186 patients in Europe, the US and Asia (ClinicalTrials.gov ID: NCT01900158).

Acknowledgments

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Conflict of Interest

Jörg Trojan: Amgen, AstraZeneca, Bayer Healthcare, Bristol Myers-Squibb, Eisai, Ipsen, Merck Serono, Merck Sharp & Dome, Lilly Imclone, Roche Servier (C/A), Ipsen, Roche (RF); **Stefan Kasper:** Incyte, Servier (C/A), Servier (H), Bristol-Myers Squibb (RF); **Dan Palmer:** Bristol-Myers Squibb, Sirtex, Roche, Eisai (C/A), Bristol-Myers Squibb, Sirtex, Bayer, Nucana (RF); **Pål K. Selbo:** Oslo University Hospital (IP); **Hans Olivecrona:** PCI Biotech AS (E); **Lena Finnesand:** PCI Biotech AS (E, OI); **Anders Høgset:** PCI Biotech AS (E, OI), Inventor on a patent on using photchemical internalization for the treatement of cholangiocarcinoma (IP); **Per Walday:** PCI Biotech AS (E, OI). The other authors indicated no financial relationships.

(C/A) Consulting/advisory relationship; (RF) Research funding; (E) Employment; (ET) Expert testimony; (H) Honoraria received; (OI) Ownership interests; (IP) Intellectual property rights/inventor/patent holder; (SAB) Scientific advisory board

Data Availability

The data underlying this article will be shared on reasonable request to the corresponding author.

References

1. Sultan AA, Jerjes W, Berg K, et al. Disulfonated tetraphenyl chlorin (TPCS2a)-induced photochemical internalisation of bleomycin in patients with solid malignancies: a phase 1, dose-escalation, first-in-man trial. *Lancet Oncol*. 2016;17(9):1217-1229.
2. Witzigmann H, Berr F, Ringel U, et al. Surgical and palliative management and outcome in 184 patients with hilar cholangiocarcinoma: palliative photodynamic therapy plus stenting is comparable to r1/r2 resection. *Ann Surg*. 2006;244(2):230-239.
3. Cheon YK, Lee TY, Lee SM, Yoon JY, Shim CS. Longterm outcome of photodynamic therapy compared with biliary stenting alone in patients with advanced hilar cholangiocarcinoma. *HPB (Oxford)*. 2012;14(3):185-193.
4. Lu Y, Liu L, Wu JC, Bie LK, Gong B. Efficacy and safety of photodynamic therapy for unresectable cholangiocarcinoma: a meta-analysis. *Clin Res Hepatol Gastroenterol*. 2015;39(6):718-724.
5. Wentrup R, Winkelmann N, Mitroshkin A, et al. Photodynamic therapy plus chemotherapy compared with photodynamic therapy alone in hilar nonresectable cholangiocarcinoma. *Gut Liver*. 2016;10(3):470-475.
6. Hauge T, Hauge PW, Warloe T, et al. Randomised controlled trial of temoporfin photodynamic therapy plus chemotherapy in nonresectable biliary carcinoma – PCS Nordic study. *Photodiagnosis Photodyn Ther*. 2016;13:330-333.
7. Valle J, Wasan H, Palmer DH, et al.; ABC-02 Trial Investigators. Cisplatin plus gemcitabine versus gemcitabine for biliary tract cancer. *N Engl J Med*. 2010;362(14):1273-1281.
8. Okusaka T, Nakachi K, Fukutomi A, et al. Gemcitabine alone or in combination with cisplatin in patients with biliary tract

cancer: a comparative multicentre study in Japan. *Br J Cancer.* 2010;103(4):469-474.
9. Lamarca A, Ross P, Wasan HS, et al. Advanced Intrahepatic Cholangiocarcinoma: Post Hoc Analysis of the ABC-01, -02, and -03 Clinical Trials. *J Natl Cancer Inst.* 2020;112(2):200-210.
10. Ortner ME, Caca K, Berr F, et al. Successful photodynamic therapy for nonresectable cholangiocarcinoma: a randomized prospective study. *Gastroenterology.* 2003;125(5):1355-1363.
11. Moole H, Tathireddy H, Dharmapuri S, et al. Success of photodynamic therapy in palliating patients with nonresectable cholangiocarcinoma: a systematic review and meta-analysis. *World J Gastroenterol.* 2017;23(7):1278-1288.
12. Hong MJ, Cheon YK, Lee EJ, Lee TY, Shim CS. Long-term outcome of photodynamic therapy with systemic chemotherapy compared to photodynamic therapy alone in patients with advanced hilar cholangiocarcinoma. *Gut Liver.* 2014;8(3):318-323.
13. Pereira SP, Jitlal M, Duggan M, et al. PHOTOSTENT-02: porfimer sodium photodynamic therapy plus stenting versus stenting alone in patients with locally advanced or metastatic biliary tract cancer. *ESMO Open.* 2018;3(5):e000379.
14. Wiedmann M, Caca K, Berr F, et al. Neoadjuvant photodynamic therapy as a new approach to treating hilar cholangiocarcinoma: a phase II pilot study. *Cancer.* 2003;97(11):2783-2790.
15. Berg K, Nordstrand S, Selbo PK, Tran DT, Angell-Petersen E, Høgset A. Disulfonated tetraphenyl chlorin (TPCS2a), a novel photosensitizer developed for clinical utilization of photochemical internalization. *Photochem Photobiol Sci.* 2011;10(10):1637-1651.
16. Dechêne A, Kasper S, Olivecrona H, Schirra J, Trojan J. Photochemical internalization and gemcitabine combined with first-line chemotherapy in perihilar cholangiocarcinoma: observations in three patients. *Endosc Int Open.* 2020;8(12):E1878-E1883.

Figures and Tables

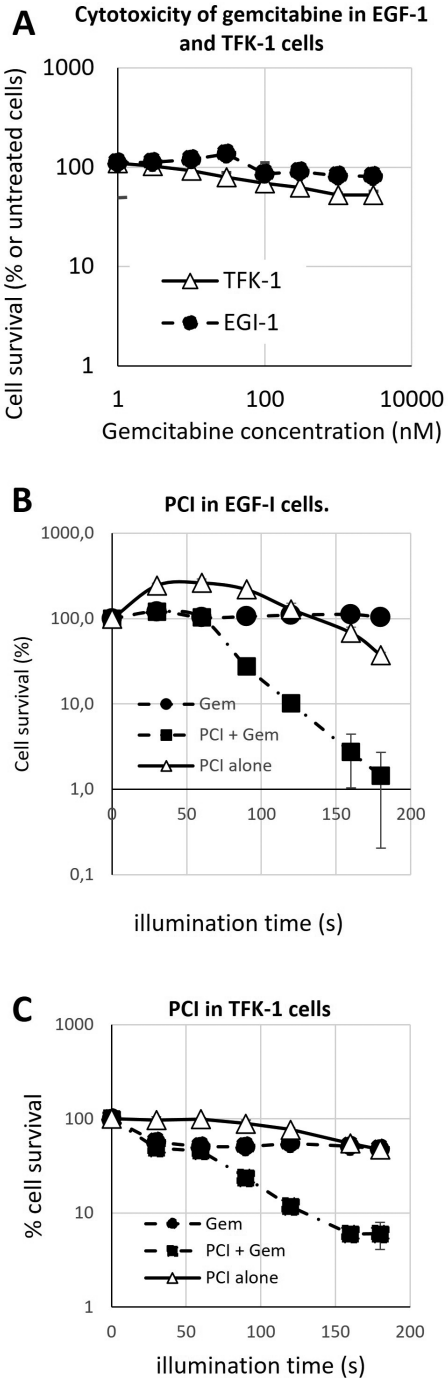

Figure 3. Preclinical studies with PCI and gemcitabine. Viability of CCA cell lines TFK-1 and EGI-1 were analyzed by the MTT assay as described. Data points are mean values of three parallel measurements (±standard deviation) and represent one representative of three independent experiments. (**A**) Cytotoxicity of gemcitabine without PCI. (**B**). Photochemical internalization with 100 nM gemcitabine in TFK-1 cells. (**C**) Photochemical internalization with 100 nM gemcitabine in EGI-1 cells.

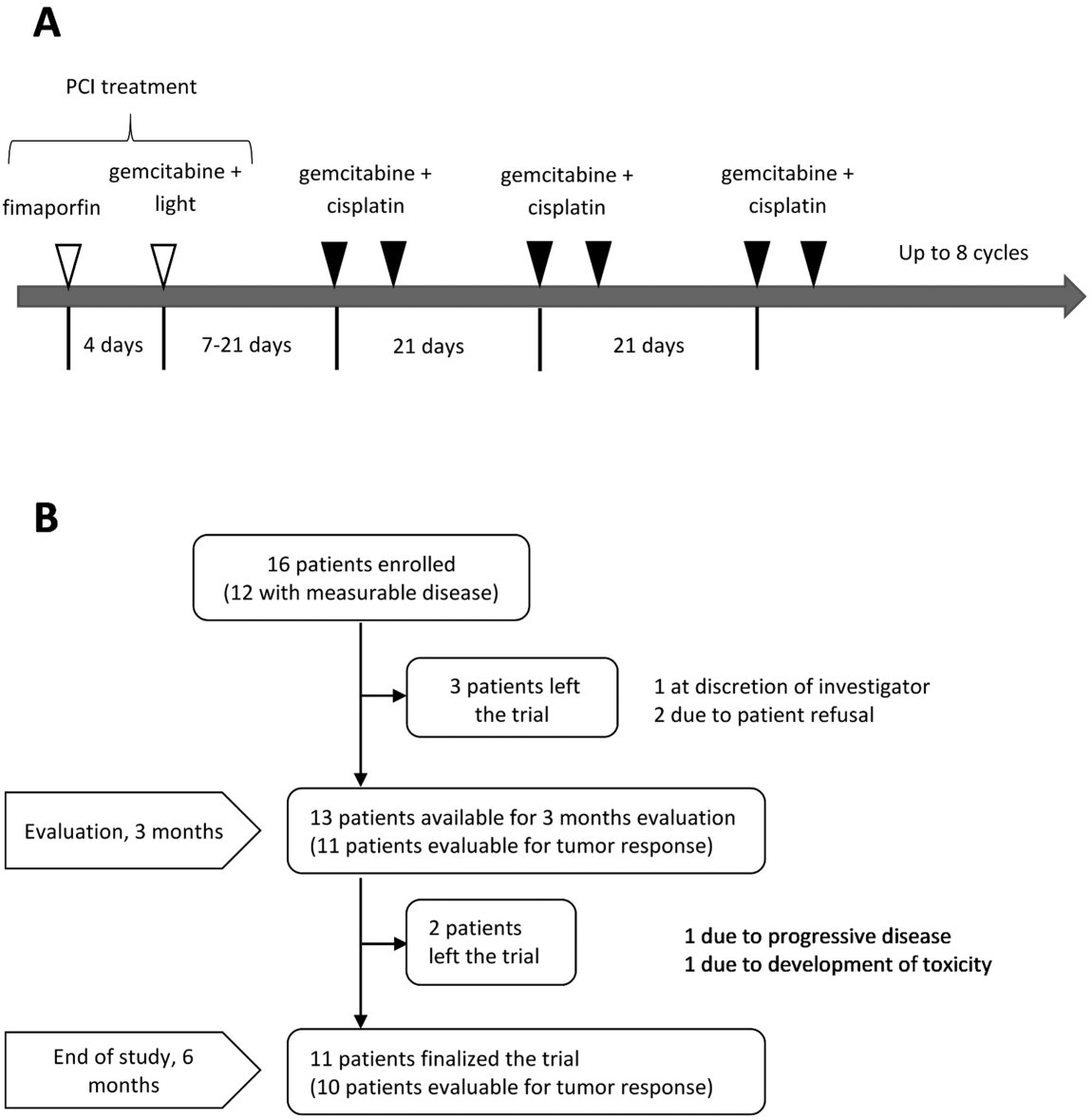

Figure 4. Study design and patient disposition. (**A**) Overall design of the clinical study (for details, see Materials and Methods). Patients were administered fimaporfin on day 0, and on day 4 gemcitabin was administered, followed by tumor illumination 3-4 hours later. Gem/cis chemotherapy was commenced 7-21 days after illumination and was given for up to eight cycles. (**B**) Disposition of patients in the study.

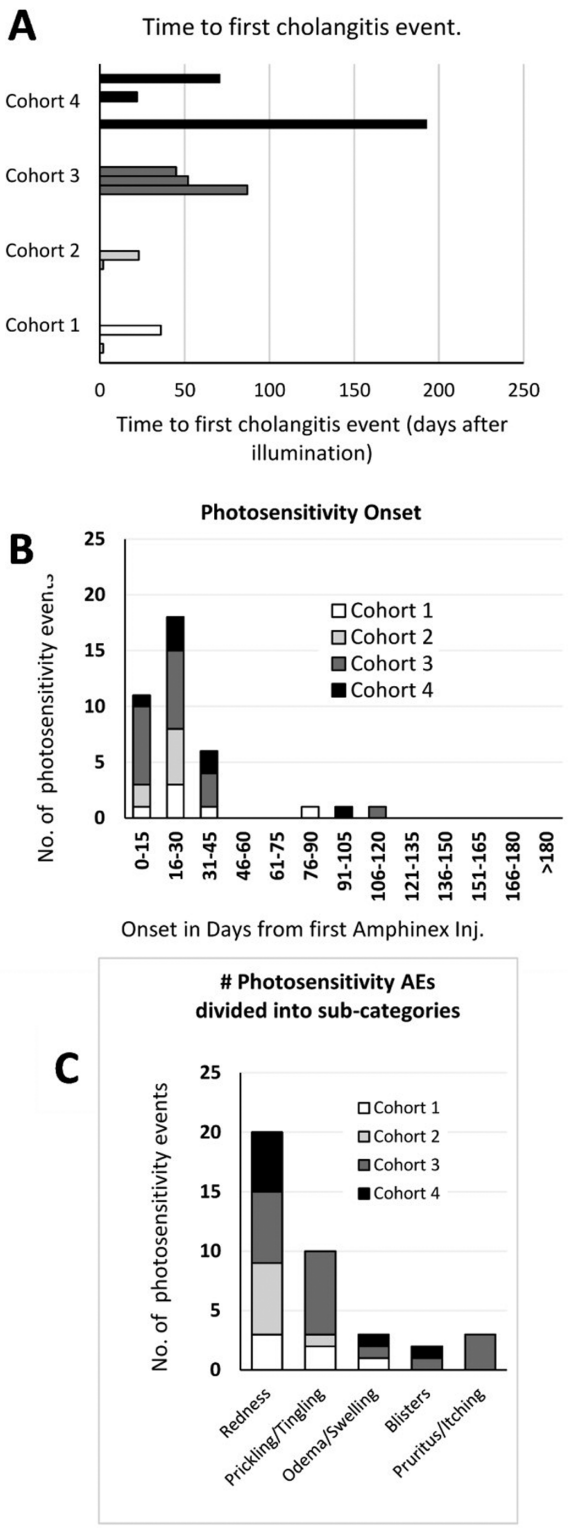

Figure 5. Cholangitis and photosensitivity events. (**A**) Time to first cholangitis event. In cohort 4, there were 3 patients not having cholangitis events; the same was the case for 1 patient in each of the cohorts 1, 2, and 3. (**B**) Timing of the onset of photosensitivity events. (**C**) Types of photosensitivity events in the different cohorts.

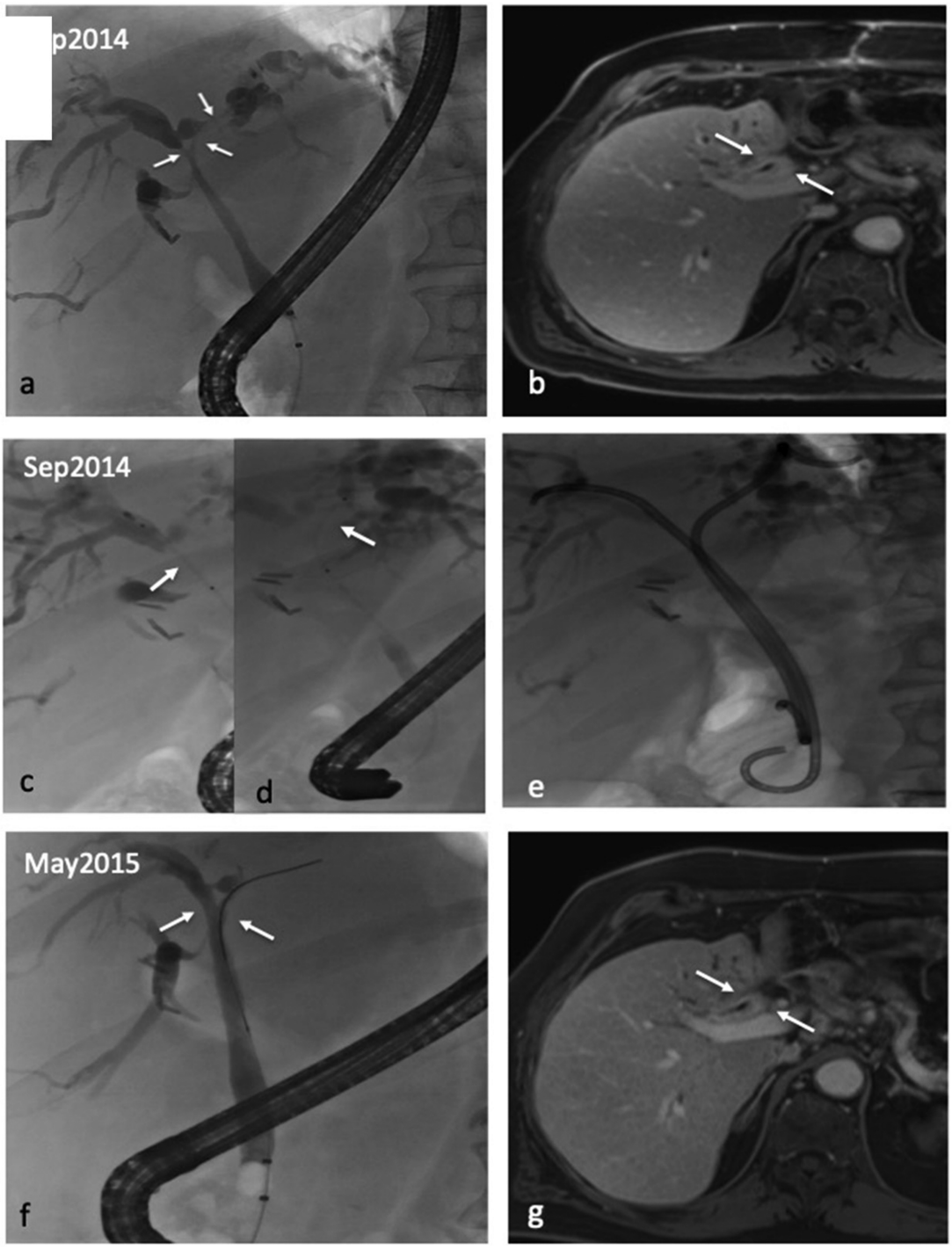

Figure 6. Effect of PCI treatment in a patient with hilar cholangiocarcinoma (cohort 2) (**A**) Fluoroscopic imaging of the hilar stenosis with dilated intrahepatic bile ducts at study entry; (**B**) Thickening of the central bile duct wall in corresponding MRI; (**C**, **D**) Fluoroscopic imaging of the cylindrical light diffuser in the right (**C**) and left (**D**) main hepatic bile ducts. (**E**) Biliary drainage with plastic stents. (**F**) Fluoroscopic imaging of the hilar region with only a minimal residual stenosis in the main left bile duct at the end of treatment; (**G**) unchanged thickening of the central bile duct wall in corresponding MRI.

Table 1. Overall safety evaluation (percentage of patients having a specific event/total number of events).

Adverse event	Cohort 1 (n = 3)		Cohort 2 (n = 3)		Cohort 3 (n = 4)		Cohort 4 (n = 6)		Total (n = 16)	
	Grade 1/2	Grade 3/4	Grade 1/2	Grade 3/4	Grade 1/2	Grade 3/4	Grade 1/2	Grade 3/4	Grade 1/2	Grade 3/4
Cardiac	0	0	33%/1	0	0	0	17%/2	17%/2	13%/3	6%/2
Gastrointestinal	100%/11	33%/1	100%/5	0	50%/2	50%/3	67%/11	17%/1	63%/29	25%/5
Infections	33%/1	33%/1	0	0	25%/1	25%/1	67%/6	0	38%/8	13%/2
Respiratory	33%/3	0	0	0	25%/1	0	0	17%/1	13%/4	7%/1
Hematological	33%/2	33%/4	33%/5	33%/1	50%/3	75%/6	83%/18	50%/10	56%/28	50%/21
Neutropenia	0	33%/4	33%/2	33%/1	25%/1	75%/5	17%/4	50%/6	19%/7	50%/16
Thrombocytopenia	33%/2	0	0	0	0	0	33%/4	17%/1	19%/6	6%/1
Leukopenia	0	0	33%/3	0	25%/1	25%/1	67%/7	33%/3	38%/11	19%/4
General disorders	67%/10	0	67%/7	0	50%/2	0	100%/11	0	75%/30	0
Pyrexia	67%/3	0	67%/4	0	50%/2	0	67%/5	0	63%/14	0
Fatigue	33%/4	0	33%/1	0	0	0	17%/1	0	19%/6	0
Hepatobiliary	33%/3	33%/1	33%/1	67%/2	75%/6	75%/7	17%/1	0	19%/6	0
Cholangitis	67%/3	33%/1	0	67%/2	25%/4	75%/6	0	50%/4	19%/7	56%/13
Icterus	0	0	33%/1	0	0	0	0	0	6%/1	0
Cholestasis	0	0	0	0	0	25%/1	0	0	0	6%/1
Biliary infection	0	0	0	0	25%/1	0	0	0	6%/1	0
Liver abscess and biliary sepsis	0	0	0	0	0	0	0	17%/1	0	6%/1
Skin and subcutaneous	100%/6	0	67%/7	0	75%/24	25%/2	67%/10	0	75%/47	6%/2

Table 2. Overall survival data.

Overall survival, months	Cohort 1 (n = 3)	Cohort 2 (n = 3)	Cohort 3 (n = 4)	Cohort 4 (n = 6)	Total
Mean	18.8	28.4	12.1	22.0	20.1
Median	13.8	23.8	14.1	22.8	15.4
Range	9.4-33.3	14.1-47.3	2.6-17.5	3.2-45[a]	2.6-47.4

[a]Patient still alive at 45 months.

Table 3. Doses and patient characteristics in the different cohorts in the clinical study.

Cohort	1	2	3	4	All
Fimaporfin dose, mg/kg	0.06	0.06	0.12	0.25	
Light dose, J/cm	15	30	30	30	
Number of patients	3	3	4	6	16
ECOG performance	0: 100%	0: 67%	0: 100%	0: 83%	0: 87.5%
	1:	1:33%	1:	1: 17%	1:16.5%
Number of patients with measurable disease	2	2	3	5	10
Target lesion size (longest diameter, cm), median/range	2.35/1.5-3.2	2.80/1.9-3.7	3.60/1.9-7	4.60/2.1-7.8	3.65/1.5-7.8

Table 4. Patient characteristics, treatment response, and survival.

Cohort	Age, years	Gender	TNM stage	3 months	6 months	Survival, months
1	58	F	T3N0M0	SD	PD	9.4
1	63	F	T2Bn2M0	SD	SD	13.8
1	65	M	T2bN0M0	NE	NE	33.3*
2	73	M	T1N2M0	NE	NE	14.1
2	65	M	T0N1M0	SD	SD	23.8
2	61	M	T4N0M0	SD	SD	47.3
3	77	F	TxNxM0	NE	—	2.6
3	78	M	T2N1M0	SD	SD	17.5
3	72	M	T4N1M0	PR	PR	16.1
3	64	M	T3N1M0	CR	CR	12.1
4	57	M	T2N0M0	NE	PD	8.5
4	61	M	T1N1M0	PR	PR	30.9
4	51	M	T3N1M0	SD	—	14.7
4	65	M	T1N0M0	PR	CR	35.1
4	73	M	T3N2MX	NE	—	3.2
4	47	M	TxNxM1	SD	PR	45**

Characteristics of tumors, response, and survival in individual patients.
*Patient evaluated as tumor free at 33 months.
**Patient was still alive at manuscript submission.
Abbreviations: CR, complete response; NE, not evaluable; PD, progressive disease; PR, partial response; SD, stable disease; TNM, tumor node metastasis.