Received: 4 July 2023 | Revised: 22 September 2023 | Accepted: 26 September 2023

DOI: 10.1111/cts.13661

CLINICAL TRIAL IN PROGRESS

Protocol of the IntenSify-Trial: An open-label phase I trial of the CYP3A inhibitor cobicistat and the cytostatics gemcitabine and nab-paclitaxel in patients with advanced stage or metastatic pancreatic ductal adenocarcinoma to evaluate the combination's pharmacokinetics, safety, and efficacy

Nicolas Hohmann[1] | **Martin Ronald Sprick**[2,3] | **Moritz Pohl**[4] | **Azaz Ahmed**[1] | **Jürgen Burhenne**[5] | **Marietta Kirchner**[4] | **Lucian Le Cornet**[6] | **Markus Kratzmann**[6] | **Jacek Hajda**[7] | **Albrecht Stenzinger**[8] | **Karen Steindorf**[9] | **Stefan Delorme**[10] | **Heinz-Peter Schlemmer**[10] | **Sabine Riethdorf**[11] | **Ron van Schaik**[12] | **Klaus Pantel**[11] | **Jens Siveke**[13,14] | **Thomas Seufferlein**[15] | **Dirk Jäger**[1,16] | **Walter E. Haefeli**[5] | **Andreas Trumpp**[2,3,17] | **Christoph Springfeld**[1]

Correspondence
Christoph Springfeld, Department Medical Oncology, Heidelberg University Hospital, National Center for Tumor Diseases, Im Neuenheimer Feld 460, 69120 Heidelberg, Germany.
Email: christoph.springfeld@med.uni-heidelberg.de

Abstract

Expression of CYP3A5 protein is a basal and acquired resistance mechanism of pancreatic ductal adenocarcinoma cells conferring protection against the CYP3A and CYP2C8 substrate paclitaxel through metabolic degradation. Inhibition of CYP3A isozymes restores the cells sensitivity to paclitaxel. The combination of gemcitabine and nab-paclitaxel is an established regimen for the treatment of metastasized or locally advanced inoperable pancreatic cancer. Cobicistat is a CYP3A inhibitor developed for the pharmacoenhancement of protease inhibitors. The addition of cobicistat to gemcitabine and nab-paclitaxel may increase the antitumor effect. We will conduct a phase I dose escalation trial with a classical 3 + 3 design to investigate the safety, tolerability,

Abbreviations: AE, adverse event; AUC, area under the curve; CLsys, systemic clearance; C_{max}, peak concentration; CYP, cytochrome P450; DLT, dose limiting toxicity; eCLmet, estimated metabolic clearance; EOS, end of study; MTD, maximum tolerable dose; NCA, non-compartemental Analysis; ORR, objective response rate; OS, overall survival; PDAC, pancreatic ductal adenocarcinoma; PFS, progression-free survival; PRO, patient-reported outcomes; QoL, quality of life; RDE, recommended dose for expansion; $t_{1/2}$, terminal half-life; UPLC-MS/MS, ultraperformance liquid chromatography coupled tandem mass spectrometry; V_{ss}, volume of distribution at steady-state.

Nicolas Hohmann and Martin Ronald Sprick contributed equally to this work.

Andreas Trumpp and Christoph Springfeld contributed equally to this work.

For Affiliation refer page on 8.

[Correction added on 13 November2023, after first online publication: Moritz Pohl was omitted in error and has been added as co-author in this version.]

and pharmacokinetics (PKs) of gemcitabine, nab-paclitaxel, and cobicistat. Although the doses of gemcitabine (1000 mg/m^2) and cobicistat (150 mg) are fixed, three dose levels of nab-paclitaxel (75, 100, and 125 mg/m^2) will be explored to account for a potential PK drug interaction. After the dose escalation phase, we will set the recommended dose for expansion (RDE) and treat up to nine patients in an expansion part of the trial. The trial is registered under the following identifiers EudraCT-Nr. 2019-001439-29, drks.de: DRKS00029409, and ct.gov: NCT05494866. Overcoming resistance to paclitaxel by CYP3A5 inhibition may lead to an increased efficacy of the gemcitabine and nab-paclitaxel regimen. Safety, efficacy, PK, and RDE data need to be acquired before investigating this combination in a large-scale clinical study.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

Gemcitabine with nab-paclitaxel is a standard chemotherapy for metastatic pancreatic cancer, but all patients sooner or later develop resistance, leading to progressive disease and finally death. Intratumoral CYP3A expression has been identified as a potential resistance mechanism in preclinal studies. Cobicistat is an inhibitor of CYP3A and licensed to enhance the plasma concentration of HIV protease inhibitors.

WHAT QUESTION WILL THE STUDY ADRESS?

We hypothesize that co-administration of cobicistat with gemcitabine and nab-paclitaxel will counteract basal and acquired chemotherapy resistance, thereby improving chemotherapy efficacy.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

This open-label phase I trial will investigate safety, tolerability and pharmacokinetics of the gemcitabine, nab-paclitaxel and cobicistat combination. A dose escalation phase with classical 3+3 design will determine the recommended dose for an expansion phase. The efficacy of the chemotherapy combination in terms of response rate, progression-free survival, and overall survival will also be analyzed and compared to historical data for gemcitabine with nab-paclitaxel alone.

HOW MAY THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

If the combination of cobicistat, gemcitabine and nab-paclitaxel turns out to be tolerable and safe and also has promising efficacy, further randomized study can be conducted and potentially improve systemic therapy of pancreatic cancer.

INTRODUCTION

Despite recent advances in therapy and the use of new treatment regimens, such as 5-Fluorouracil, folic acid, irinotecan, oxaliplatin (FOLFIRINOX), gemcitabine/nab-paclitaxel, or 5-FU/nal-irinotecan, the prognosis of advanced-stage pancreatic cancer is grim, with a 5-year overall survival (OS) of only 2.7%.[1] Although these treatments result in response or disease stabilization in many patients, ultimately all patients experience progression of disease due to drug-resistant cell clones.[2–7]

Drug-metabolizing enzymes of the cytochrome P450 (CYP) family have an established role as determinants of systemic exposure of small-molecule anti-cancer agents.[8] As such, they are drivers of intra- and interpatient variability in pharmacokinetics (PKs) and hence pharmacodynamics.[9] Expression of CYP isozymes in tumor tissue has been reported since the 1990s but recent works suggest a functional role as intracellular elimination mechanism of anticancer drugs conferring resistance to their antineoplastic effect.[10] CYP3A5 expression in pancreatic adenocarcinoma (PDAC) cells of the exocrine-like

subtype is associated with basal and acquired resistance toward antineoplastic CYP3A substrates. Moreover, cells of the classical and the quasi-mesenchymal subtypes that do not initially express CYP3A5 can acquire CYP3A5 expression upon treatment with a drug that is inactivated by CYP3A5.[10] Using that mechanism, tumor cells with drug-metabolizing capacity degrade active drug possibly also mediating resistance of neighboring tumor cells not expressing significant levels of CYP3A5. The cytoskeleton-targeting anticancer agent paclitaxel, the tyrosine kinase inhibitor erlotinib, and the topoisomerase-inhibiting irinotecan are susceptible to degradation by CYP3A5-expressing tumor cells.[10] Hence, inhibition of CYP3A-mediated drug metabolism by tumor cells is a possible strategy to overcome basal and acquired drug resistance and increase efficacy of anticancer agents that are CYP3A substrates.

In CYP3A5-expressing adenocarcinoma of the pancreas, lower concentrations of anti-neoplastic CYP3A substrates are necessary to kill tumor cells when co-administered with a CYP3A inhibitor.[10–12] Therefore, addition of a CYP3A inhibitor to the established pancreatic cancer regimen of gemcitabine and nab-paclitaxel may lead to an improved efficacy in terms of prolonged progression-free survival (PFS) and OS.[13] Cobicistat is a CYP inhibitor developed as a pharmacological enhancer of protease inhibitors for the treatment of human immunodeficiency virus infections and has a favorable safety profile.[14]

Because the systemic elimination of paclitaxel depends on CYP3A4, CYP3A5, and CYP2C8, the risk of the combination lies in a potential PK drug interaction that would result in exaggerated paclitaxel exposure. There are no clinical drug interaction data between a CYP3A inhibitor and nab-paclitaxel. However, there is one interaction study between the strong CYP3A inhibitor ketoconazole and solvent-based paclitaxel in ovarian cancer in which no significant changes in clearance were observed.[15] A physiology-based PK model of solvent-based paclitaxel predicted a 1.7-fold increase in paclitaxel area under the curve (AUC) when adding a CYP3A inhibitor.[16] Increased paclitaxel exposure may lead to more severe adverse drug reactions, most notably neutropenia and peripheral neuropathy are possible. The severity of solvent-based paclitaxel neurotoxicity is related to increased AUC and peak plasma concentration, and the presence of the CYP3A4*22 single nucleotide polymorphism, which is associated with decreased CYP3A activity in vivo.[17]

Solvent-based paclitaxel and nab-paclitaxel considerably differ in their PK characteristics.[18] No drug interaction data neither from human trials nor in silico modeling is available for nab-paclitaxel. Cobicistat may increase the efficacy of nab-paclitaxel in the treatment of pancreatic cancer. Therefore, it is necessary to investigate the novel combination of gemcitabine, nab-paclitaxel, and cobicistat – all three marketed drugs with a known safety profile.

METHODS/DESIGN

We designed a phase I clinical trial to determine safety, tolerability, PKs, and antineoplastic activity of gemcitabine, nab-paclitaxel, and cobicistat that will enroll a total of 18 patients with advanced stage, inoperable, or metastatic pancreatic ductal adenocarcinoma. Eligibility criteria are given in detail in the supplementary material (Table S1). The open-label trial consists of a classical 3+3 dose escalation part.[19] to determine the maximum tolerable dose (MTD) of nab-paclitaxel and/or the recommended dose for expansion (RDE) of nab-paclitaxel followed by an expansion part at RDE (Figure 1).

We received approval from the national competent authority (Bundesinstitut für Arzneimittel) and the responsible institutional review board (Ethikkommission der Medizinischen Fakultät Heidelberg). The trial is registered under EudraCT no. 2019-001439-29 and in the German Clinical Trials Register ("Deutsches Register Klinischer Studien") under DRKS00029409, and Clinicaltrials.gov under NCT05494866.

This is an exploratory trial. In the escalation part, a minimum of three and a maximum of 18 patients will be enrolled as described by the escalation algorithm (Figure 1). The maximum number of 18 patients was set for practical reasons and there was no formal sample size calculation for the dose escalation part. Dropouts that do not complete the first cycle for other reasons than experiencing a dose limiting toxicity (DLT) will be replaced.

In addition, a formal calculation of the sample size for the expansion part of this trial was not possible because the investigated treatment combination has never been used in patients and therefore no reasonable assumptions can be made about its effect. Actually, the sample size of the expansion part depends on the number of patients in the escalation part and is chosen so that there are a total of 18 evaluable patients in the entire trial.

Dose escalation part

The dose escalation part of the study enrolls patients who would receive gemcitabine and nab-paclitaxel as a palliative treatment according to the local standard of care. Treatment is administered in sequentially opened cohorts. The patients will be treated with ascending doses of i.v. nab-paclitaxel (dose level 1: 75 mg/m^2; dose level 2: 100 mg/m^2; dose level 3: 125 mg/m^2) and with 1000 mg/m^2 of i.v. gemcitabine on days 1, 8, and 15 of a 28-day

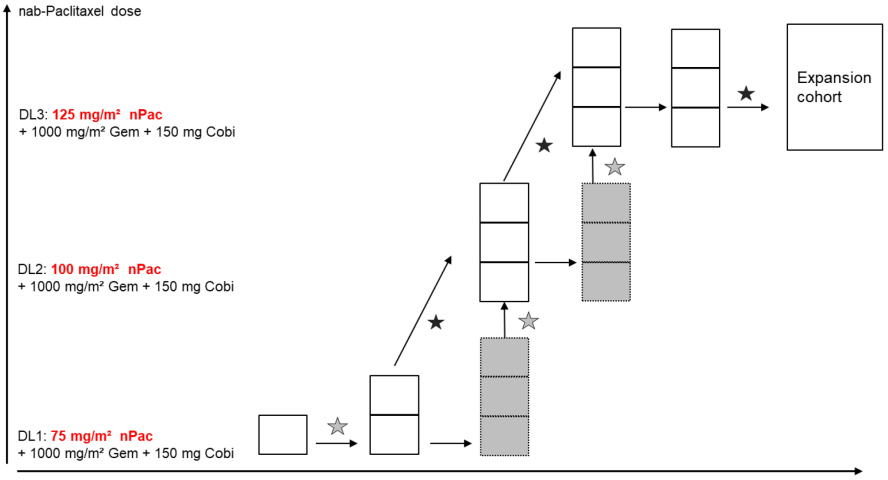

FIGURE 1 The dose escalation part encompasses three sequential cohorts with three (+3) patients each treated with gemcitabine, nab-paclitaxel, and cobicistat. The starting dose of nab-paclitaxel for cohort 1 will be 75 mg/m^2. Inclusion of additional blocks (shaded in gray) of three patients per cohort depends on the number of DLT occurrences in the first three patients. Escalation of subsequent dosing (i.e., opening of cohorts with escalated doses) also depends on the occurrence of DLTs. The data monitoring committee (DMC meetings, represented by black stars) will review safety data when data post 28 days of treatment of at least three patients per cohort are available (C1-DLT observation period) and then make a recommendation on opening of the next cohorts with an escalated dose or not. DLT, dose limiting toxicity; DMC, data monitoring committee.

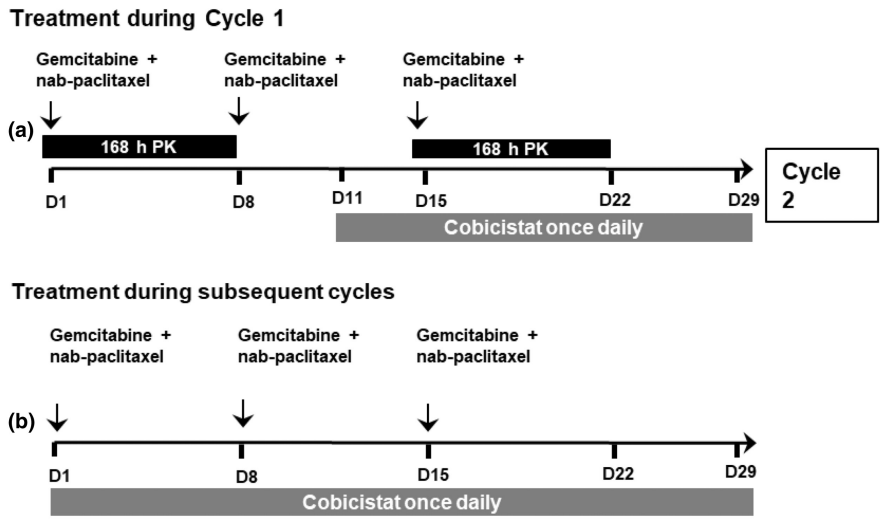

FIGURE 2 During cycle 1 (a) patients will be treated with nab-paclitaxel and gemcitabine at days 1, 8, and 15. Cobicistat will be added starting with day 11. Blood samples for nab-paclitaxel pharmacokinetics (PKs) will be collected at day 1 and day 15 over 48 h and before the next nab-paclitaxel dosing (~168 h postdose). Treatment during subsequent cycles (b) will consist of cobicistat once daily (q.d.) with gemcitabine and nab-paclitaxel at days 1, 8, and 15 in all patients until progression.

cycle combined with 150 mg of oral cobicistat once daily on days 11–28 of cycle 1 and days 1–28 in the following cycles (Figure 2). A 10 µg dose of i.v. midazolam will be administered at cycle 1 day 1 and cycle 1 day 15 for CYP3A phenotyping. Each cohort will enroll three patients at first. In the first cohort, the first patient will be a forerunner and must have completed the first cycle before the next two patients may be enrolled. The first 28 days of treatment are the observation period for DLT. After each cohort of three (+3), patients' data will be reviewed by the data monitoring committee (DMC), which will then advise on how to proceed. If no DLT occurs in the first three patients, the next dose level will be opened. If one DLT occurs, three more patients will be added to the cohort and if no additional DLT occurs in the overall group of six patients, the next dose level starts as well. If two or more DLTs occur in the first three patients or the group that was expanded to six patients, the trial will be halted. In this case, the DMC will meet and advise whether a protocol amendment is required and the study can be resumed or after completion of cohort 2 to define MTD and/or RDE and start the expansion part.

The DMC will convene when the third patient and, if applicable, the sixth patient of each cohort has reached day 1 of cycle 2 and safety data from cycle 1 is fully available, allowing a recommendation on dose escalation or transition to the expansion part. If the study is set on hold, no more patients must be included. Patients already enrolled

in the trial and well tolerating the trial medication may continue treatment. After completion of the dose escalation part, a recommendation of the DMC will be necessary to decide whether or not to open the dose expansion cohort and which nab-paclitaxel RDE will be used.

After setting the RDE and opening the dose expansion part, intrapatient dose escalation will be allowed. An increase of the dose is at the investigator's discretion and will only be made in patients who tolerated the combination well so far.

Dose expansion part

Similar patients with identical eligibility criteria as in the escalation part will be eligible for the dose expansion part. All patients of the expansion part may be enrolled and treated in parallel. Patients will receive 1000 mg/m^2 of gemcitabine and the RDE of nab-paclitaxel, as determined during dose escalation, on days 1, 8, and 15. In the first cycle, cobicistat 150 mg once daily will be added to the nab-paclitaxel/gemcitabine regimen starting on day 11. A 10 µg dose of i.v. midazolam will be administered at cycle 1 day 1 and cycle 1 day 15 for CYP3A phenotyping. During the subsequent cycles, the patients will take 150 mg of cobicistat orally, continuously from day 1 to day 28 of each cycle. The dose levels of gemcitabine and nab-paclitaxel for individual patients may have to be reduced according to individual tolerability at their treating physician's discretion. Within the treatment phase of the study (12 months), each patient is planned to receive up to 12 treatment cycles, unless treatment will be discontinued or interrupted.

After start of treatment, patients will be staged according to the local standard of care in intervals of ~8–12 weeks (based on treating physician's discretion) until completion of treatment or until radiological progression of disease according to RECIST 1.1 or the physician's decision to stop treatment for medical reasons, such as intolerance or clinical progression of disease. Safety will be observed as from the first administration of study medication until 4 weeks after end of treatment. The end of safety follow-up determines the end of study (EOS) for the individual patient. For patients alive at the EOS, a 3-monthly survival follow-up will be performed subsequent to the EOS visit.

Study-related procedures

After giving written informed consent, patients will undergo a screening consisting of a physical examination, measurement of vital signs (pulse, blood pressure, and body temperature), a 12-lead electrocardiogram, and blood drawings for laboratory assessments (clinical chemistry, blood cell count including differential, coagulation, serology for human immunodeficiency virus, hepatitis B virus, and hepatitis C virus). Patients will provide a urine sample and a baseline liquid biopsy sample and a whole blood sample for pharmacogenomics from germline will be drawn. Patients will undergo a computed tomography (CT) or magnetic resonance imaging (MRI) scan of the thorax, abdomen, and pelvis or submit a CT scan available from routine clinical practice not older than 28 days. During the screening phase, biopsy of a tumor site is mandatory.

The patient will come to the trial center on days 1, 2, 3, 8, 15, 16, 17, and 22 of the first cycle. Nab-paclitaxel and gemcitabine are administered on days 1, 8, and 15. Before each treatment day, a blood cell count, clinical chemistry, and physical examination will be performed to assess whether the treatment can be given or not. Treatment with cobicistat starts on cycle 1 day 11. In addition to the antineoplastic treatment, patients will receive an i.v. bolus of 10 µg of midazolam on cycle 1 day 1 and cycle 1 day 15. Blood will be drawn before and 0.5, 1, 2, 2.5, 3, 4, and 6 h. Radiological response to treatment will be assessed by CT or MRI scans, performed in routine intervals of 8 to 12 weeks according to the local standard of care. The scans will be evaluated according to RECIST 1.1.[20]

Primary objective/end point

The primary objective is to establish a safety and tolerability profile of the drug combination. The primary end point of the trial is the safety of the combination as measured by the incidence of DLTs up to week 4 of treatment during dose escalation. A DLT is defined as any adverse event (AE) or clinically significant abnormal laboratory value of Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 grade 3 or higher up to 28 days after the first dose of nab-paclitaxel with a suspected causal relationship to the study medication, but must be unrelated to the disease, disease progression, an inter-current illness, or co-medication and must not fall under the exceptions listed in Table S2. The occurrence of DLTs during the safety observation period determines the DMC's recommendation to open subsequent cohorts. Safety will be further (apart from the primary end point) evaluated by the incidence of treatment-emergent AEs (NCI CTCAE version 5.0).

Secondary objectives/end points

To collect data on the antitumor activity of the combination in relation to radiologic response to treatment as measured through by CT of the thorax, and CT or MRI

scans of the abdomen, and pelvis every 8–12 weeks. The scans will be evaluated according to RECIST 1.1 criteria and we will report PFS, disease control rate, objective response rate (ORR), and duration of response. Further efficacy data will be acquired in terms of OS, defined as the time from start of treatment to time of death from any cause. Administrative censoring is applied for patients who survive to the end of the trial.

We will investigate changes in patient-reported outcomes (PROs), including quality of life (QoL), during treatment with nab-paclitaxel in combination with gemcitabine and cobicistat. PROs are assessed by questionnaires and are collected at screening and at day 1 of each cycle during and after treatment. The PRO end points are the absolute scores at all assessed time points as well as the changes from the screening visit. The following PRO scores will be calculated according to the actual scoring manuals: the European Organization for Research and Treatment of Cancer Quality of Life Questionnaire (EORTC QLQ)-C30 Health-related QoL summary score[21]; the EORTC QLQ-C30 and EORTC PAN26 function and symptom scores[22,23]; fatigue from the EORTC QLQ-FA12[24]; sleep problems from the Pittsburgh Sleep Quality Index (PSQI)[25]; perceived cognitive impairments and impact of cognitive changes from the FACT-cog[26]; anxiety and depression from the PHQ-4.[27]

For all patients in both study parts, nab-paclitaxel plasma concentrations from sequentially drawn blood samples at cycle 1 day 1, and cycle 1 day 15, will be measured by ultra-performance liquid chromatography coupled to tandem mass spectrometry (UPLC-MS/MS). The plasma concentration-time profile will be constructed from those concentrations. The following standard PK parameters will be calculated by noncompartmental analysis (NCA): observed area under the plasma concentration-time curve ($AUC_{0\text{-}72}$), AUC extrapolated to infinity ($AUC_{0\text{-}inf}$), observed peak plasma concentration (C_{max}), systemic clearance (CL_{sys}) of nab-paclitaxel, terminal elimination half-life ($t_{1/2}$), volume of distribution (V_D), and volume of distribution at steady-state (V_{ss}). In addition, midazolam plasma concentrations will be measured in plasma from sequentially drawn blood samples at C1D1, and C1D15 by UPLC-MS/MS and the time-plasma concentration profile will be constructed.[28] The following PK parameters will be calculated through NCA: $AUC_{0\text{-}inf}$, $AUC_{2\text{-}4}$, $AUC_{0\text{-}24}$, C_{max}, CL_{sys}, $t_{1/2}$, V_D, V_{ss}, and CL_{sys}. The estimated metabolic clearance of midazolam (eCL_{met}) is a derived marker for CYP3A activity based on a limited sampling strategy.[9,29,30]

PK samples are only collected during the first cycle. For dose-exposure-response analysis, we will correlate the AUC of paclitaxel when administered with cobicistat during cycle 1 with the occurrence of AEs leading to dose reduction. We will also correlate paclitaxel AUC when administered with cobicistat with PFS and OS. Because we explore different dose levels with three to six patients each, the case number may not be sufficient when accounting for dose reductions. Therefore, we will still calculate the cumulative gemcitabine and paclitaxel doses of all patients and correlate the average dose per cycle with PFS, OS, and occurrence of AEs in an exploratory manner.

Exploratory objectives

On day 1 of each cycle, a liquid biopsy sample will be collected to analyze cell-free DNA for copy-number variations, mutations, and methylation patterns. Additionally, circulating tumor cells (CTCs) will be detected using the CellSearch system (Menarini Silicon Biosystems) to evaluate CTC numbers. These analyses have the aim to develop novel biomarkers that predict drug resistance and treatment response from the blood samples.

The trial requires sampling of a fresh pretreatment tumor biopsy and a biopsy upon progression of disease. From these, fresh material and formalin fixed paraffin embedded slides will be generated to analyze PDAC.[31] In addition, immunostaining and fluorescence in situ hybridization for CYP3A5 and ABCB1 expression/amplification in the tumor will be performed, including evaluation of further biomarkers associated with PDAC subtypes, progression, drug resistance, and treatment response. We aim to also generate organoid cultures from biopsy samples with the unique opportunity to obtain paired samples from the same patient before and after progression. These models will be used to test for drug resistance mechanism to identify novel therapeutic targets and biomarkers. Genetic, epigenetic, and mRNA expression changes associated with drug resistance, PDAC progression, and aggressiveness from organoid cultures will be determined.

All patients will have their CYP3A5 and CYP2C8 genotypes determined from germline to analyze the contribution of CYP3A5 expressor versus non-expressor status and CYP2C8 genotype on nab-paclitaxel PKs with and without concomitant administration of the CYP3A inhibitor cobicistat.

Statistical analysis

The primary end point of the escalation part, the occurrence of a DLT in a patient, will be summarized by frequencies in the escalation part population which includes all patients of the escalation part who start

treatment and were not replaced. All secondary end points are analyzed descriptively according to their scale in the full analysis population including all patients of the escalation and expansion part who were not replaced. In accordance with an intention-to-treat approach a termination of the study treatment is handled by a treatment policy approach which means that the end points are used regardless of the early termination. For time-to-event end points, Kaplan–Meier plots will be presented and median survival with 95% confidence intervals will be given. Subgroup analysis will be conducted for dose groups and first-line treatment. The PK end points will be summarized by comprehensive summary statistics in the PK set consisting of all patients enrolled in the dose escalation and expansion part with all PK data available from day 1 and day 15 of cycle 1. The PK analysis will additionally be conducted grouped by CYP3A5. All analyses will be conducted with SAS (version 9.4 or higher). A statistical analyses plan describing the planned analysis in more detail was finalized before first patient in the study.

DISCUSSION

This will be the first trial exploring the combination of a CYP3A inhibitor with nab-paclitaxel to improve response to treatment—a concept similar to the addition of beta-lactamase inhibitors to penicillin-derivatives for the treatment of bacterial infection.

We expect a considerable scientific gain from this trial in the following areas:

(1) This clinical trial is a proof-of-concept trial investigating the inhibition of a newly identified resistance factor in pancreatic cancer. The data generated in this trial are needed to decide whether a larger clinical trial exploring a combination therapy of gemcitabine, dose-adjusted nab-paclitaxel, and cobicistat with the standard regimen of gemcitabine and nab-paclitaxel is feasible and potentially beneficial.
(2) No PK drug interaction trials with a CYP3A inhibitor were conducted with nab-paclitaxel. The trial will provide the clinical data on drug interaction between nab-paclitaxel and cobicistat, thus adding to knowledge in the field of oncological drug safety. This quantitative data is necessary for improved clinical decision making when treating patients with PDAC.
(3) Analyzing tumor biopsies collected before and after therapy with gemcitabine and nab-paclitaxel will grant further insight into tumor biology, especially with regard to the baseline and post-treatment rate of CYP3A5 expression in advanced-stage or metastatic PDAC.

The nab-paclitaxel dose and the resulting exposure when combined with cobicistat is key to this trial, especially for safety but also for the scientific interpretation of the results. There is no drug interaction data for nab-paclitaxel when combined with a CYP3A inhibitor, but a drug interaction trial conducted with solvent-based paclitaxel and the CYP3A inhibitor ketoconazole detected no change in plasma concentrations of paclitaxel or its 6-α-hydroxy metabolite.[15] A recently published physiology-based PK (PBPK)-pharmacodynamic model of solvent-based paclitaxel estimated an increased AUC ratio of 1.7 for solvent-based paclitaxel exposure when combining a single 175 mg/m^2 dose of paclitaxel with ketoconazole.[16] CYP-mediated biotransformation is the main elimination route for paclitaxel. Therefore, the elimination rate constant λ_z of both nab-paclitaxel and sb-paclitaxel was nearly identical,[32] we therefore reasoned that despite the differences in systemic exposure and distribution, changes in elimination and thus the extent of the drug interaction due to CYP-inhibition will be similar for nab-paclitaxel. The PK data acquired in this trial will be useful to further inform modeling and simulation approaches.

Adapting the standard nab-paclitaxel dose of 125 mg/m^2 to the calculated 1.7-fold increase from the PBPK model[16] would correspond to a dose of 73.5 mg/m^2. We have therefore set the starting dose to 75 mg/m^2, which corresponds to the dose reduction step 2 in the MPACT trial[5] and the Abraxane SmPC.[33] A subanalysis of the efficacy and safety-profile of the phase III MPACT trial showed that dose reductions are common: 46% had greater than or equal to one nab-paclitaxel dose reduction and 74% had greater than or equal to one nab-paclitaxel dose delay.[34] For nab-paclitaxel, the toxicities that most commonly led to dose modifications were neutropenia, peripheral neuropathy, thrombocytopenia, and fatigue.[34,35] Of the patients who needed a dose reduction, in 60%, the dose was reduced by one step (i.e., 100 mg/m^2) and in the remaining 40% the dose was reduced by two steps (i.e., 75 mg/m^2).[35]

The need of dose modifications did not impair the efficacy of the treatment. Greater treatment exposure in terms of treatment duration, and number of administered cycles were associated with improved outcome rather than the amount of drug administered per dose.[35] Similar observations have been made for nab-paclitaxel monotherapy in metastatic breast cancer, where dose and OS did not correlate.[36]

Lower starting doses of nab-paclitaxel in combination with gemcitabine for the treatment of PDAC have been investigated in smaller clinical trials. In the phase I dose escalation trial to investigate the combination, starting doses of 100, 125, and 150 mg/m^2 were used.[2,5] Twenty patients were enrolled in the 100 mg/m^2 dosing group, 44 patients were enrolled in the 125 mg/m^2 group, and three patients

in the 150 mg/m^2 group. Efficacy of the 100 mg/m^2 group was comparable to 125 mg/m^2 with an ORR of 45% versus 48%.[2] A clinical phase II trial conducted in an elderly population with reduced dose steps of 100, 75, and 50 mg/m^2 combined with gemcitabine d1,8,15 q4w showed similar efficacy as reported in the MPACT trial.[37]

In our trial, the dosing steps in the dose escalation phase will be 75, 100, and 125 mg/m^2, which corresponds to the doses used as dose reduction steps during the phase I dose escalation and the MPACT trial.[2,5] The increase of nab-paclitaxel dose by 25 mg/m^2 per dosing step corresponds to a 33% and 25% dose increase. Bayesian approaches are considered advantageous, especially for first-in-human trials to determine MTD. We still decided for a classical 3 + 3 design[19] over Bayesian optimal interval method or a Bayesian logistic regression model with overdose control.[38] Because, here, we explore a novel combination of known drugs with already explored dose—exposure relationships and safety—the benefit of such a design would not have outweigh the increase in necessary resources and reduced ease of use and understanding of more complicated designs.

We chose the dose of 125 mg/m^2 as last dosing step, which is the standard starting dose of nab-paclitaxel when combined with gemcitabine for the treatment of PDAC. Safety and efficacy of this dose have been established through the phase III MPACT trial.[8] The phase I dose escalation trial established 125 mg/m^2 as MTD for the combination of gemcitabine and nab-paclitaxel.[2] If safe and tolerable, we aim to escalate the nab-paclitaxel dose up to 125 mg/m^2.

Intrapatient dose escalation will ensure that, once safety of the combination has been established, patients from cohorts 1 and 2 may be treated with the same dose density as patients enrolled in the dose expansion arm. This will reduce the risk of potential underexposure, once the RDE has been set. This trial is a phase I trial with safety and tolerability as primary end points. Patients of all dose levels will be pooled for analysis of efficacy, hence, the impact of intrapatient dose escalation on efficacy analysis is minimal.

No changes in PKs are expected for gemcitabine by introducing cobicistat to the regimen, because gemcitabine is degraded through non-CYP-dependent pathways.[39] Cobicistat does not exhibit severe toxicity on its own. Therefore, we will use the doses of 1000 mg/m^2 with dose reduction steps to 800 and 600 mg/m^2 that have been established as safe and efficacious when combined with nab-paclitaxel for the treatment of PDAC.[2,5,39] We will use the dose of 150 mg oral daily as per drug label for cobicistat. This dose will result in a thorough inhibition of CYP3A isozymes.[40,41] Midazolam will not be used for its medical purpose of sedation but will serve as a marker substrate for CYP3A activity[42] with and without cobicistat. For the purpose of CYP3A activity measurement, the use of microdoses that are well below the no-observed-effect level are a safe method for phenotyping CYP3A activity in patients with cancer.[43]

Whereas effects of cobicistat on hepatic and intestinal CYP isozymes are known,[13] the local distribution into the tumor and exposure of pancreatic cancer cells to cobicistat is crucial. Tissue penetration is especially important in pancreatic cancer with its high portion of desmoplastic stroma.[44] This is a limitation of the current trial as it is not designed to answer this question. We discussed measuring cobicistat in an on-treatment biopsy during steady-state but we decided against it to reduce patient burden. The clinical development of cobicistat as an enhancer of anticancer drugs in pancreatic cancer will necessitate further clinical trials to address all open questions. Tissue penetration has to be assessed in a future experiment. The best approach to reduce heterogeneity of samples would be to collect tissue of patients undergoing surgery for local pancreatic cancer (and/or metastasectomy at centers, where this procedure is deemed a treatment approach in oligometastasized pancreatic cancer) after a single pre-operative dose of cobicistat and to measure tissue penetration through imaging mass spectrometry. The first step in the clinical development of this novel combination is to obtain data on safety, tolerability, and PKs. The Intensify trial has been designed to obtain these.

In summary, the risk for a participant of this clinical trial is known and manageable, especially because approved drugs and doses are used. They will receive treatment with an inhibitor of CYP3A—eliminating a specific chemoresistance mechanism and potentially increasing or restoring the effectiveness of nab-paclitaxel treatment. Another benefit for patients with CYP3A5 negative tumors is also conceivable, because CYP3A5 may be acquired as a resistance factor during treatment.[8]

AUTHOR CONTRIBUTIONS

N.H., A.T., M.R.S., M.P., A.A., J.B., M.Ki., L.L., M.Kr., J.H., A.S., K.S., S.D., H.-P.S., S.R., R.S., K.P., J.S., T.S., D.J., W.E.H., and C.S. wrote the manuscript. N.H., A.T., M.R.S., C.S., W.E.H., D.J., M.Kr., and L.L. performed the research. N.H., M.S., M.Ki., M.P., C.S., and A.T. analyzed the data. A.T., M.R.S., J.B., A.S., R.S., S.R., and K.P. contributed new reagents/analytical tools.

AFFILIATIONS

[1]Department of Medical Oncology, Heidelberg University Hospital, National Center for Tumor Diseases, Heidelberg, Germany
[2]Division of Stem Cells and Cancer, German Cancer Research Center (DKFZ), Heidelberg, Germany
[3]Heidelberg Institute for Stem Cell Technology and Experimental Medicine (HI-STEM gGmbH), Heidelberg, Germany

[4]Institute of Medical Biometry, University Hospital Heidelberg, Heidelberg, Germany
[5]Department of Clinical Pharmacology and Pharmacoepidemiology, Heidelberg University Hospital, Heidelberg, Germany
[6]NCT Trial Center, NCT, German Cancer Research Center (DKFZ), Heidelberg, Germany
[7]Pharmacovigilance Department, Coordination Centre for Clinical Trials (KKS), Heidelberg University Hospital, Heidelberg, Germany
[8]Institute of Pathology, University Hospital Heidelberg, Heidelberg, Germany
[9]Division of Physical Activity, Prevention and Cancer, German Cancer Research Center (DKFZ) and National Center for Tumor Diseases (NCT) Heidelberg, Heidelberg, Germany
[10]Division of Radiology, German Cancer Research Center (DKFZ), Heidelberg, Germany
[11]Institute of Tumor Biology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany
[12]Department of Clinical Chemistry, Erasmus University Medical Center, Rotterdam, The Netherlands
[13]Bridge Institute of Experimental Tumor Therapy, West German Cancer Center, University Hospital Essen, University of Duisburg-Essen, Essen, Germany
[14]Division of Solid Tumor Translational Oncology, German Cancer Consortium (DKTK Partner Site Essen) and German Cancer Research Center (DKFZ), Heidelberg, Germany
[15]Department of Internal Medicine I, Ulm University Hospital, Ulm, Germany
[16]Clinical Cooperation Unit Applied Tumor Immunity, German Cancer Research Center (DKFZ), Heidelberg, Germany
[17]German Cancer Consortium (DKTK), Heidelberg, Germany

ACKNOWLEDGMENTS

The authors would like to acknowledge the scientific input given by Prof. Dr. Marcus Jörger (St. Gallen, Switzerland) and Prof. Dr. Gerd Mikus (Heidelberg, Germany) during the early planning of this clinical trial and Prof. Dr. Uwe Fuhr (Köln, Germany) for a thorough protocol review. A.T. and J.T.S. are supported by the German Cancer Consortium (DKTK). Open Access funding enabled and organized by Projekt DEAL.

CONFLICT OF INTEREST STATEMENT

M.R.S. and A.T. are holding the patient WO/2016/045799, NOVEL METHODS FOR SUB-TYPING AND TREATING CANCER. A.S. receives honoraria for advisory boards or speaker's bureau from Agilent, Aignostics, Amgen, Astra Zeneca, Bayer, BMS, Eli Lilly, Illumina, Incyte, Janssen, MSD, Novartis, Pfizer, Qlucore, Roche, Seagen, Takeda, Thermo Fisher and grants from Bayer, BMS, Chugai, Incyte, all outside the submitted work. J.T.S. receives honoraria as consultant or for continuing medical education presentations from AstraZeneca, Bayer, Boehringer Ingelheim, Bristol-Myers Squibb, Immunocore, MSD Sharp Dohme, Novartis, Roche/Genentech, and Servier. His institution receives research funding from Abalos Therapeutics, AstraZeneca, Boehringer Ingelheim, Bristol-Myers Squibb, Celgene, Eisbach Bio, and Roche/Genentech; he holds ownership and serves on the Board of Directors of Pharma15, all outside the submitted work. T.S. receives honoraria as consultant or for continuing medical education presentations from BMS, Cantargia, Mirati, Olympus America, Inc., Pierre Fabre, Scandion, Servier and Research support from Lilly, all outside the submitted work. All other authors declared no competing interests for this work.

FUNDING INFORMATION

This trial is funded by an intramural grant of the German Cancer Research Center. The German Cancer Research Center is the sponsor of the clinical trial. Funding for the translational part has been obtained from the Dietmar Hopp Foundation.

DATA AVAILABILITY STATEMENT

Once completed, the data that support the findings of this study will be made available from DKFZ on reasonable request.

ETHICS STATEMENT

The trial received the approval from the responsible IRB (Ethikkommission der Medizinischen Fakultät Heidelberg, reference number AFmo-588/2022). All patients will give written informed consent before any study-specific procedure is carried out. All methods are performed in accordance with the relevant guidelines and regulations, i.e. Declaration of Helsinki, ICH-GCP, the applicable European directives and regulations (Directive 2001/20/EC, EU Regulation 536/2014), and the national German laws (GCP-V, AMG).

ORCID

Nicolas Hohmann https://orcid.org/0000-0001-8036-8806
Azaz Ahmed https://orcid.org/0000-0003-3000-7951
Jürgen Burhenne https://orcid.org/0000-0002-2190-1698
Marietta Kirchner https://orcid.org/0000-0003-0294-2483
Albrecht Stenzinger https://orcid.org/0000-0003-1001-103X
Jens Siveke https://orcid.org/0000-0002-8772-4778
Walter E. Haefeli https://orcid.org/0000-0003-0672-6876
Andreas Trumpp https://orcid.org/0000-0002-6212-3466
Christoph Springfeld https://orcid.org/0000-0003-3843-101X

REFERENCES

1. NCI SEERS Database. Cancer stat facts: pancreatic cancer. Accessed June 15, 2023. https://seer.cancer.gov/statfacts/html/pancreas.html. Last
2. Von Hoff DD, Ramanathan RK, Borad MJ, et al. Gemcitabine plus nab-paclitaxel is an active regimen in patients with advanced pancreatic cancer: a phase I/II trial. *J Clin Oncol*. 2011;29:4548-4554.

3. Moore MJ, Goldstein D, Hamm J, et al. Erlotinib plus gemcitabine compared with gemcitabine alone in patients with advanced pancreatic cancer: a phase III trial of the National Cancer Institute of Canada clinical trials group. *J Clin Oncol*. 2007;25:1960-1966.
4. Conroy T, Desseigne F, Ychou M, et al. FOLFIRINOX versus gemcitabine for metastatic pancreatic cancer. *N Engl J Med*. 2011;364:1817-1825.
5. Von Hoff DD, Ervin T, Arena FP, et al. Increased survival in pancreatic cancer with nab-paclitaxel plus gemcitabine. *N Engl J Med*. 2013;369:1691-1703.
6. Conroy T, Bachet JB, Ayav A, et al. Current standards and new innovative approaches for treatment of pancreatic cancer. *Eur J Cancer*. 2016;57:10-22.
7. Wang-Gillam A, Li CP, Bodoky G, et al. Nanoliposomal irinotecan with fluorouracil and folinic acid in metastatic pancreatic cancer after previous gemcitabine-based therapy (NAPOLI-1): a global, randomised, open-label, phase 3 trial. *Lancet*. 2016;387(10018):545-557.
8. Mikus G, Foerster KI. Role of CYP3A4 in kinase inhibitor metabolism and assessment of CYP3A4 activity. *Transl Cancer Res*. 2017;6:S1592-159.
9. Hohmann N, Haefeli WE, Mikus G. CYP3A activity: towards dose adaptation to the individual. *Expert Opin Drug Metab Toxicol*. 2016;12:479-497.
10. Noll EM, Eisen C, Stenzinger A, et al. CYP3A5 mediates basal and acquired therapy resistance in different subtypes of pancreatic ductal adenocarcinoma. *Nat Med*. 2016;22:278-287.
11. Thomas H. Pancreatic cancer: CYP3A5 contributes to PDAC chemoresistance. *Nat Rev Gastroenterol Hepatol*. 2016;13:188.
12. Zee YK, Goh BC, Lee SC. Pharmacologic modulation strategies to reduce dose requirements of anticancer therapy while preserving clinical efficacy. *Future Oncol*. 2012;8:731-749.
13. Hossain MA, Tran T, Chen T, Mikus G, Greenblatt D. Inhibition of human cytochromes P450 in vitro by ritonavir and cobicistat. *J Pharm Pharmacol*. 2017;69:1786-1793.
14. Deeks ED. Cobicistat: a review of its use as a pharmacokinetic enhancer of atazanavir and darunavir in patients with HIV-1 infection. *Drugs*. 2014;74:195-206.
15. Jamis-Dow CA, Pearl ML, Watkins PB, Blake DS, Klecker RW, Collins JM. Predicting drug interactions in vivo from experiments in vitro. Human studies with paclitaxel and ketoconazole. *J Am Clin Oncol*. 1997;20:592-599.
16. De Sousa MM, Hatley O, Gill KL, Rowland Yeo K, Ke AB. A physiologically based pharmacokinetic—pharmacodynamic modelling approach to predict incidence of neutropenia as a result of drug-drug interactions of paclitaxel in cancer patients. *Eur J Pharm Sci*. 2020;150:105355.
17. Scripture CD, Figg WD, Sparreboom A. Peripheral neuropathy induced by paclitaxel: recent insights and future perspectives. *Curr Neuropharmacol*. 2006;4:165-172.
18. Stage TB, Bergmann TK, Kroetz DL. Clinical pharmacokinetics of paclitaxel monotherapy: an updated literature review. *Clin Pharmacokinet*. 2018;57:7-19.
19. Storer BE. Design and analysis of phase I clinical trials. *Biometrics*. 1989;45:925-937.
20. Eisenhauer EA, Therasse P, Bogaerts J, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). *Eur J Cancer*. 2009;45:228-247. doi:10.1016/j.ejca.2008.10.026
21. Giesinger JM, Kieffer JM, Fayers PM, et al. Replication and validation of higher order models demonstrated that a summary score for the EORTC QLQ-C30 is robust. *J Clin Epidemiol*. 2016;69:79-88.
22. Fayers P, Aaronson NK, Bjordal K, et al. *EORTC QLQ-C30 Scoring Manual*. European Organisation for Research and Treatment of Cancer; 2001.
23. Fitzsimmons D, Johnson CD, George S, et al. Development of a disease specific quality of life (QoL) questionnaire module to supplement the EORTC core cancer QoL questionnaire, the QLQ-C30 in patients with pancreatic cancer. EORTC study group on quality of life. *Eur J Cancer*. 1999;35:939-941.
24. Weis J, Tomaszewski KA, Hammerlid E, et al. International psychometric validation of an EORTC quality of life module measuring cancer related fatigue (EORTC QLQ-FA12). *J Natl Cancer Inst*. 2017;109.
25. Buysse DJ, Reynolds CF, Monk TH, et al. Pittsburgh sleep quality index., in APA task force. In: Rush AJ et al., eds. *Handbook of Psychiatric Measures*. APA; 2000:678-680.
26. FACT-Cog Scoring Guidelines (Version 3), www.facit.org
27. Patient Health Questionnaire (PHQ) Screeners. INSTRUCTION MANUAL Instructions for Patient Health Questionnaire (PHQ) and GAD-7 Measures.
28. Burhenne J, Halama B, Maurer M, et al. Quantification of femtomolar concentrations of the CYP3A substrate midazolam and its main metabolite 1′-hydroxymidazolam in human plasma using ultra performance liquid chromatography coupled to tandem mass spectrometry. *Anal Bioanal Chem*. 2012;402:2439-2450.
29. Hohmann N, Kocheise F, Carls A, Burhenne J, Haefeli WE, Mikus G. Midazolam microdose to determine systemic and pre-systemic metabolic CYP3A activity in humans. *Br J Clin Pharmacol*. 2015;79:278-285.
30. Katzenmaier S, Markert C, Riedel KD, Burhenne J, Haefeli WE, Mikus G. Determining the time course of CYP3A inhibition by potent reversible and irreversible CYP3A inhibitors using a limited sampling strategy. *Clin Pharmacol Ther*. 2011;90:666-673.
31. Muckenhuber A, Berger AK, Schlitter AM, et al. Pancreatic ductal adenocarcinoma subtyping using the biomarkers hepatocyte nuclear factor-1A and Cytokeratin-81 correlates with outcome and treatment response. *Clin Cancer Res*. 2018;24:351-359.
32. Sparreboom A, Scripture CD, Trieu V, et al. Comparative preclinical and clinical pharmacokinetics of a cremophor-free, nanoparticle albumin-bound paclitaxel (ABI-007) and paclitaxel formulated in Cremophor (Taxol). *Clin Cancer Res*. 2005;11:4136-4143.
33. Bristol Myers Squibb. Abraxane 5 mg/mL powder for dispersion for infusion SmPC. 2022 [cited 2022 Aug 01]. https://www.ema.europa.eu/en/documents/product-information/abraxane-epar-product-information_en.pdf
34. Vogel A, Römmler-Zehrer J, Li JS, McGovern D, Romano A, Stahl M. Efficacy and safety profile of nab-paclitaxel plus gemcitabine in patients with metastatic pancreatic cancer treated to disease progression: a subanalysis from a phase 3 trial (MPACT). *BMC Cancer*. 2016;16:817.
35. Scheithauer W, Ramanathan RK, Moore M, et al. Dose modification and efficacy of nab-paclitaxel plus gemcitabine vs. gemcitabine for patients with metastatic pancreatic cancer: phase III MPACT trial. *J Gastrointest Oncol*. 2016;7:469-478.
36. Gradishar W. San Antonio Breast Cancer Symposium 2011:poster P5-19-13.

37. Ibusuki M, Inoue T, Kitano R, et al. Gemcitabine plus nab-paclitaxel with initial dose reduction for older patients with advanced pancreatic cancer. *J Geriatric Oncol*. 2021;12:118-121.
38. Pantoja K, Lanke S, Munafo A, et al. Designing phase I oncology dose escalation using dose–exposure–toxicity models as a complementary approach to model-based dose–toxicity models. *CPT Pharmacometrics Syst Pharmacol*. 2022;11:1371-1381.
39. Lilly. Gemzar 200 mg powder for solution for infusion SmPC. 2008 [cited 2022 Aug 01]. https://www.ema.europa.eu/en/documents/referral/gemzar-article-30-referral-annex-i-ii-iii_en.pdf
40. Sherman EM, Worley MV, Ungers NR, Gauthier TP, Schafer JJ. Cobicistat: review of a pharmacokinetic enhancer for HIV infection. *Clin Ther*. 2015;37:1876-1893.
41. European Medicines Agency. Guideline on the investigation of drug interactions. 2012. [cited 2022 Aug 01]. https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-investigation-drug-interactions-revision-1_en.pdf
42. U.S. Food and Drug Administration. Clinical Drug Interaction Studies—Cytochrome P450 Enzyme- and Transporter-Mediated Drug Interactions Guidance for Industry. 2020. [cited 2022 Aug 01]. https://www.fda.gov/media/134581/download
43. Hohmann N, Halama B, Siller N, Mikus G, Haefeli WE. Response to "can CYP3A activity be evaluated for drug interaction using a nanogram dose of probe drug?": evaluation of CYP3A activity with microdoses of midazolam. *Clin Pharmacol Ther*. 2014;95:490-491.
44. Li T, Wang YK, Khokhlova TD, et al. Pulsed high intensity focused ultrasound (pHIFU) enhances delivery of doxorubicin in a preclinical model of pancreatic cancer. *Cancer Res*. 2015;75:3738-3746.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Hohmann N, Sprick MR, Pohl M, et al. Protocol of the IntenSify-Trial: An open-label phase I trial of the CYP3A inhibitor cobicistat and the cytostatics gemcitabine and nab-paclitaxel in patients with advanced stage or metastatic pancreatic ductal adenocarcinoma to evaluate the combination's pharmacokinetics, safety, and efficacy. *Clin Transl Sci*. 2023;00:1-11. doi:10.1111/cts.13661