BMJ Open Study protocol of a phase IB/II clinical trial of metformin and chloroquine in patients with *IDH1*-mutated or *IDH2*-mutated solid tumours

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ABSTRACT

Introduction High-grade chondrosarcoma, high-grade glioma and intrahepatic cholangiocarcinoma are aggressive types of cancer with a dismal outcome. This is due to the lack of effective treatment options, emphasising the need for novel therapies. Mutations in the genes *IDH1* and *IDH2* (isocitrate dehydrogenase 1 and 2) occur in 60% of chondrosarcoma, 80% of WHO grade II–IV glioma and 20% of intrahepatic cholangiocarcinoma. *IDH1/2*-mutated cancer cells produce the oncometabolite *D*-2-hydroxyglutarate (*D*-2HG) and are metabolically vulnerable to treatment with the oral antidiabetic metformin and the oral antimalarial drug chloroquine.

Methods and analysis We describe a dose-finding phase Ib/II clinical trial, in which patients with *IDH1/2*-mutated chondrosarcoma, glioma and intrahepatic cholangiocarcinoma are treated with a combination of metformin and chloroquine. Dose escalation is performed according to a 3+3 dose-escalation scheme. The primary objective is to determine the maximum tolerated dose to establish the recommended dose for a phase II clinical trial. Secondary objectives of the study include (1) determination of pharmacokinetics and toxic effects of the study therapy, for which metformin and chloroquine serum levels will be determined over time; (2) investigation of tumour responses to metformin plus chloroquine in *IDH1/2*-mutated cancers using CT/MRI scans; and (3) whether tumour responses can be measured by non-invasive *D*-2HG measurements (mass spectrometry and magnetic resonance spectroscopy) of tumour tissue, serum, urine, and/or bile or next-generation sequencing of circulating tumour DNA (liquid biopsies). This study may open a novel treatment avenue for *IDH1/2*-mutated high-grade chondrosarcoma, glioma and intrahepatic cholangiocarcinoma by repurposing the combination of two inexpensive drugs that are already approved for other indications.

Ethics and dissemination This study has been approved by the medical-ethical review committee of the Academic Medical Center, Amsterdam, The Netherlands. The report will be submitted to a peer-reviewed journal.

Strengths and limitations of this study

- To the best of our knowledge, this is the first clinical trial that investigates the combination of metformin and chloroquine in patients with cancer.
- Tumour responses to the study therapy will be monitored using conventional CT/MRI scans and using magnetic resonance spectroscopy or serum mass spectrometry for *D*-2-hydroxyglutarate levels.
- Because this is primarily a dose-finding study, we may not be able to study the efficacy of metformin and chloroquine.
- When patients do not consent to tumour biopsies/re-resections, this diminishes the possibility for translational analyses.

Trial registration number This article was registered at ClinicalTrials.gov identifier (NCT02496741): Pre-results.

INTRODUCTION

IDH1 and *IDH2* (isocitrate dehydrogenase 1 and 2) are homodimeric enzymes that reversibly convert isocitrate to α -ketoglutarate (α KG) in cytoplasm and mitochondria, respectively. Somatic heterozygous mutations in *IDH1/2* that produce the oncometabolite *D*-2-hydroxyglutarate (*D*-2HG) are observed in substantial percentages of various tumour types such as chondrosarcoma (60%), WHO grade II–III glioma (80%), secondary WHO grade IV glioblastoma (80%) and intrahepatic cholangiocarcinoma (20%).¹ In addition, *IDH1/2* mutations occur in varying percentages of acute lymphocytic leukaemia (10%), acute myeloid leukaemia (AML; 20%), angioimmunoblastic T cell lymphoma (40%), colorectal cancer (5%) and melanoma (12%).¹ In chondrosarcoma and glioma, *IDH1/2* mutations are considered

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very early or even inaugural genetic defects, and are thus present in a large fraction of, or even all, cancer cells.^{2 3} This renders *IDH1/2* mutations an interesting target for anticancer treatment because such tumour homogeneity decreases the risk of therapy resistance.⁴ Recently, inhibitors of mutant *IDH1* and *IDH2* were developed that may be effective in stalling malignant progression of early-stage *IDH1/2*-mutated cancers.^{5 6}

Prognosis and therapeutic options of cancers in which *IDH1/2* mutations occur

The prognosis of solid tumours with frequent occurrence of *IDH1/2* mutations remains poor. The current standard therapy for chondrosarcoma is surgery. There is no evidence for a benefit of (adjuvant) radiotherapy or chemotherapy, as chondrosarcoma is considered to be highly therapy resistant.⁷ Consequently, the 1-year survival rate of metastasised high-grade chondrosarcoma is <10%.⁸ Gliomas vary from WHO grade II diffuse astrocytoma and diffuse oligodendroglioma, with median survivals of more than 5 years,⁹ to WHO grade IV glioblastoma, with a median survival of only 15 months despite aggressive treatment using radiotherapy and temozolomide.¹⁰ Gliomas are diffusely growing tumours, which render surgery ineffective, emphasising the dire need for novel therapies. Furthermore, the blood–brain barrier (BBB) prohibits the use of most chemotherapeutics and the surrounding normal brain hampers aggressive radiotherapy regimens due to limitations that are raised by healthy brain tissue.¹¹ Intrahepatic cholangiocarcinoma is resectable in only 40% of patients.¹² In unresectable cases, patients with intrahepatic cholangiocarcinoma are offered palliative treatment as standard of care with the chemotherapy combination of cisplatin and gemcitabine, with a median overall survival of 11.7 months.¹³

Metabolic effects of *IDH1/2* mutations

Heterozygous hotspot *IDH1/2* mutations disable *IDH1/2* wild-type enzyme activity^{14–16} and induce a neoenzymatic activity that leads to the production and subsequent accumulation of *D-2HG*.^{17–19} *D-2HG* is normally present only in trace amounts in normal tissues and cells but accumulates up to 50 mM in *IDH1/2*-mutated glioma.¹⁷ *D-2HG* is chemically very similar to α KG and inhibits over 60 α KG-dependent enzymes, resulting in global DNA/histone hypermethylation, decreased hypoxia-inducible factor 1 α expression and perturbed collagen maturation.¹ Depending on the cellular context, these effects are the basis of oncogenesis and imply a dependence on *D-2HG* of early-stage *IDH1/2*-mutated tumours.¹

IDH1/2-mutated cancer cells need α KG to synthesise *D-2HG* and fuel the tricarboxylic acid (TCA) cycle to support their metabolism. α KG is generated by glycolysis (glucose breakdown) or glutaminolysis (glutamine/glutamate breakdown).²⁰ *IDH1/2* mutations downregulate α KG levels by consuming α KG and by inhibition of α KG production via direct effects, that is, by disabling *IDH1/2* wild-type kinetics, and indirect effects, for example, by

decreasing TCA cycle activity.¹ Therefore, *IDH1/2*-mutated cancer cells rely on glutaminolysis for sufficient α KG supply to generate the oncometabolite *D-2HG* (figure 1).²¹ The conversion of glutamate to α KG is catalysed by glutamate dehydrogenase, which is the final step of glutaminolysis and can be inhibited by the anti-malaria drug chloroquine and the antidiabetic drug metformin.^{20 22–24} In addition, *IDH1*-mutated glioma cells show increased levels of autophagy, likely as a survival mechanism of cells to metabolic stress by catabolising proteins in order to provide substrates for energy production in stress/starvation contexts.²⁵ Autophagy is inhibited by chloroquine²⁶ and the anticancer properties of chloroquine may thus be selective for *IDH1/2*-mutated cells because it inhibits glutaminolysis and autophagy on which the cells are dependent.

IDH1/2 mutations induce further metabolic stress in *IDH1/2*-mutated cancer cells via inhibition of the TCA cycle and electron transport chain (ETC) by *D-2HG*. More specifically, *D-2HG* inhibits enzymatic activity of complex IV (cytochrome C oxidase) of the ETC²⁷ and the TCA(-like) enzymes *IDH1/2* and α KG dehydrogenase.¹⁶ This reduces oxidative phosphorylation, the primary source of ATP in cancer cells.^{27 28} This metabolic stress is amplified in vitro in *IDH1*-mutated glioma and colorectal carcinoma cells using compounds that inhibit ETC complex I, such as the oral antidiabetic biguanides metformin and phenformin, which selectively restrict the proliferation of these cells.^{16 28}

Another metabolic vulnerability of *IDH1/2*-mutated glioma may be their excess deposition of the acidic *D-2HG* in their microenvironment, which is hypothesised to contribute to their diffuse growth.^{29 30} Chloroquine buffers the tumour milieu³¹ and may decrease this acidification, reduce the diffuse growth and ultimately increase the treatability of *IDH1/2*-mutated glioma.

Metabolism of *IDH1/2*-mutated tumours as therapeutic target

Metformin and chloroquine increase metabolic stress in *IDH1/2*-mutated cells, as is described above. Patients with *IDH1/2*-mutated glioblastoma have a prolonged survival and better radiotherapy/chemotherapy response when compared with *IDH1/2* wild-type counterparts,^{14 32 33} while in chondrosarcoma a correlation between mutation and survival was absent.² We and others have shown that *IDH1/2* mutations sensitise glioma and colorectal carcinoma cells to therapies that involve oxidative stress, such as radiotherapy, cisplatin and carmustine.^{16 34 35} Combined, these data suggest that at least some types of cancer with *IDH1/2* mutations should be targeted by compounds that exploit this presumed metabolic vulnerability rather than compounds that decrease metabolic stress (ie, *IDH1/2*-mutant inhibitors). Accordingly, we hypothesised that the difference in survival of patients with *IDH1/2*-mutated glioma or intrahepatic cholangiocarcinoma versus *IDH1/2* wild-type counterparts is caused by dysregulation of cellular defence mechanisms

Figure 1 Cellular carbohydrate and glutamine metabolism showing the pathways in which wild-type IDH1 and IDH2 are functional. Isocitrate dehydrogenase 1, 2 and 3 (IDH1/2/3) catalyse the conversion of isocitrate to α -ketoglutarate (α KG) in the cytoplasm and mitochondria, respectively, where IDH1 and IDH2 are NADPH producing and IDH3 is NADH producing. This reaction occurs in tricarboxylic acid (TCA) cycle(-like) metabolism and is fed by glucose influx and/or glutamine influx. The conversion of glutamine into α KG occurs in the glutaminolysis pathway and the last step is catalysed by glutamate dehydrogenase (GDH), which is inhibited by chloroquine and metformin. The TCA cycle produces NADH, which is used in the electron transport chain (ETC) and oxidative phosphorylation to generate ATP. Complex I of the ETC is inhibited by metformin. Wild-type IDH1/2 (IDH1/2^{WT}) differs from mutant IDH1/2 (IDH1/2^{MUT}) because the latter enzyme converts α KG into a novel oncometabolite, D-2-hydroxyglutarate (D-2HG). CoA, coenzyme A; NADPH, nicotinamide adenine dinucleotide phosphate.

by *IDH1/2* mutations against anticancer therapy.^{1 16 36} Little is known about the role of *IDH1/2* mutations in late-stage cancer. It is plausible that with increasing mutational burden, the dependence of late-stage malignant tumours on *IDH1/2* mutations decreases, diminishing the therapeutic index of *IDH1/2*-mutant inhibitors.^{37 38} On the other hand, metabolic stress that results from *IDH1/2* mutations persists, and this metabolic vulnerability provides an excellent target for therapy irrespective of the tumour stage.

Discussion regarding the use of metformin and chloroquine

Metformin and chloroquine are readily available, inexpensive and safe drugs that are already FDA/EMA (U.S. Food and Drug Administration/European Medicines Agency) approved for other indications. The safety profiles of metformin and chloroquine are favourable over other anticancer modalities, which may aid rapid implementation of these drugs into therapies for patients with *IDH1/2*-mutated cancers. A caveat is that the combined safety of metformin and chloroquine is to be proven by our study, although there are no reports of toxic side effects of this combination in the literature whereas the prevalence of both diabetes and malaria is high. Since both drugs are off patent, combination treatment with metformin and chloroquine can become a therapeutic advance for patients with *IDH1/2*-mutated solid tumours that is considerably less expensive than products of other

anticancer research efforts. The potential of metformin and chloroquine as adjuvant drugs was recently demonstrated in vivo, where metformin or chloroquine had a sensitising and/or synergistic antitumour effect in combination with temozolomide,^{39 40} cisplatin^{41 42} and gemcitabine^{43 44} in xenograft models or proof-of-concept clinical trials of various types of human cancer, including glioma. Metformin, but not chloroquine, sensitised xenograft models of various types of human cancer to ionising radiation.^{45 46}

Possible concerns may be related to the bioavailability of metformin. We have observed high expression of metformin transporters in chondrosarcoma, glioma, and intrahepatic cholangiocarcinoma cell lines and primary tissue (OCT1-3; The Cancer Cell Line Encyclopedia⁴⁷ and our own unpublished data). Therefore, we expect to achieve sufficient intratumoural metformin concentrations with dose levels 2 and 3 of our dose-escalation protocol. Whereas millimolar metformin concentrations are necessary to activate the necessary antineoplastic cellular targets in vitro, these targets were already activated at ± 300 -fold lower metformin concentrations in vivo. When metformin fails to show any metabolic or antitumour effect, we may investigate the feasibility of phenformin treatment in future studies. Phenformin is the lipophilic analogue of metformin which does not depend on transporters to enter cells. However, phenformin has a

less favourable safety compared with metformin because it carries an increased risk of inducing lactic acidosis. As a consequence, phenformin approval for the treatment of diabetes mellitus type 2 was withdrawn by the FDA and EMA in the 1970s⁴⁸ and in contrast to metformin, phenformin is not readily available.

With respect to chloroquine, possible concerns may be related to the plethora of cellular targets of chloroquine. Inhibition of autophagy and glutaminolysis and buffering of the tumour milieu are the potential therapeutic targets of chloroquine in *IDH1/2*-mutated cancers. Besides these, chloroquine also induces apoptosis and affects the body's immune response to the tumour in vitro and/or in vivo at concentrations that may be achieved using the dose that we use in the present clinical trial.³¹ These properties of chloroquine as a 'dirty drug' may lead to toxicity problems.

For the treatment of glioma, adequate drug penetration of the BBB is necessary for relevant tumour responses. Notwithstanding that high-grade glioma often destructs the BBB, in vivo experiments in mice have shown that metformin and chloroquine adequately pass the BBB.^{49 50}

Non-invasive detection of *IDH1/2* mutations

The gold standard of *IDH1/2* mutation detection is genetic analysis of tumour DNA. In glioma, 90% of all *IDH1/2* mutations are *IDH1*^{R132H} and its presence can be reliably detected using an immunohistochemistry of glioma tissue with an *IDH1*^{R132H}-specific antibody.⁵¹ The presence of *IDH1/2* mutations in AML⁵² and intrahepatic cholangiocarcinoma⁵³ can be easily, reliably and non-invasively detected via determination of 2HG levels or *D*-2HG levels in serum or urine by mass spectrometry (MS). Furthermore, MS-determined 2HG serum levels correlate with therapy response in these cancers.^{52 53} In a previous study investigating intrahepatic cholangiocarcinoma, total 2HG levels in serum predicted the presence of an *IDH1/2* mutation (as determined using targeted DNA sequencing) with a sensitivity of 83% and a specificity of 90%.⁵³

Whereas no non-invasive detection methods of *IDH1/2* mutations have been described to be effective in chondrosarcoma yet, the presence of *IDH1/2* mutations in glioma can be determined using magnetic resonance spectroscopy (MRS) of the brain, which detects intratumoural 2HG levels.^{54 55} Conversely, serum 2HG levels correlate poorly with the *IDH1/2* mutational status in glioma due to a limited BBB passage of *D*-2HG.⁵⁶ Urine 2HG levels are higher in patients with *IDH1*-mutated glioma than in patients with *IDH1* wild-type glioma,⁵⁷ although another study reported decreased 2HG levels in the urine of patients with *IDH1*-mutated glioma and showed that the ratio of serum 2HG levels to urine 2HG levels is most predictive for the *IDH1* mutational status in glioma.⁵⁸ Most aforementioned measurements determined total 2HG levels and thus did not discriminate between the *D*-enantiomer of 2HG (which is specific for *IDH1/2* mutations) and the *L*-enantiomer of 2HG (which is unspecific

and is generated during hypoxia).^{59 60} Better separation of *D*-2HG and *L*-2HG may allow for *IDH1/2* mutational status predictions with higher sensitivity and specificity.

Besides methods that detect *D*-2HG accumulation, *IDH1/2* mutations may also be detected via next-generation sequencing (NGS) of circulating tumour DNA (ctDNA) that is isolated from serum as liquid biopsies. Liquid biopsies contain a collection of ctDNA sequences which is representative for the heterogeneity of the tumour. Therefore, liquid biopsies are more informative than tissue biopsies, which are subject to selection bias as a result of the tumour heterogeneity. In liquid biopsies, variant allelic frequencies can be used as biomarkers for tumour load and dynamic clonal hierarchies within the tumour.⁶¹

Hypothesis and outlook

To summarise, fundamental and translational research by us and others revealed that *IDH1/2* mutations impart therapeutically targetable metabolic vulnerabilities to cells from several types of cancer.^{16 20 21 27 28} We aim to use these metabolic alterations in *IDH1/2*-mutated tumours for screening purposes and tumour response monitoring purposes using non-invasive modalities. Furthermore, we aim to specifically inhibit the metabolic processes that are essential to *IDH1/2*-mutated tumours using metformin and chloroquine, which specifically target the metabolic vulnerabilities that are caused by *IDH1/2* mutations.

We hypothesise that metformin and chloroquine can be safely used as anticancer drugs for patients with *IDH1/2*-mutated chondrosarcoma, glioma and intrahepatic cholangiocarcinoma and that tumour response to treatment can be monitored by measuring tumour size and/or levels of *D*-2HG in serum, urine, bile and/or the tumoural mass. This hypothesis will be tested in a phase Ib/II clinical trial. There are no reports of clinical trials of combined treatment with metformin and chloroquine yet. In the future, metformin and chloroquine may be used as stand-alone therapy for patients with *IDH1/2*-mutated cancers, especially in chondrosarcoma for which no effective therapies beside surgery exist, or besides conventional anticancer treatments such as radiation and temozolomide in glioma and cisplatin and gemcitabine in intrahepatic cholangiocarcinoma.

METHODS AND ANALYSIS

Overall study design

Metformin and chloroquine in *IDH1/2*-mutated solid tumours (MACIST) is a non-randomised, open-label, dose-finding, multicentre phase Ib/II clinical trial with a combined regimen of metformin and chloroquine in patients with *IDH1/2*-mutated chondrosarcoma, glioma or intrahepatic cholangiocarcinoma. Drug dosing will follow a 3+3 dose escalation scheme. Patients will be enrolled at three academic hospitals in The Netherlands (Academic Medical Centre and VU University Medical

Centre, both in Amsterdam, and the Leiden University Medical Centre in Leiden).⁶²

Objectives

Primary objective

To determine the maximum tolerated dose (MTD) and recommended dose (RD) of metformin plus chloroquine in patients with *IDH1/2*-mutated chondrosarcoma, glioma or intrahepatic cholangiocarcinoma.

Secondary objectives

- ▶ to describe the toxic effects and pharmacokinetics of metformin plus chloroquine in patients with *IDH1/2*-mutated chondrosarcoma, glioma or intrahepatic cholangiocarcinoma;
- ▶ to provide evidence of complete or partial tumour regression in patients with *IDH1/2*-mutated chondrosarcoma, glioma or intrahepatic cholangiocarcinoma after treatment with metformin plus chloroquine;
- ▶ to provide evidence that the *IDH1/2* mutational status of chondrosarcoma, glioma and intrahepatic cholangiocarcinoma can be assessed using enantiomer-specific measurements that determine the separate *D*-2HG and *L*-2HG levels in serum, urine or bile (with better sensitivity and specificity than with measurements that determine total 2HG concentrations);
- ▶ to provide evidence that the *IDH1/2* mutational status of patients with chondrosarcoma and intrahepatic cholangiocarcinoma can be determined by MRS-facilitated detection of intratumoural 2HG levels or liquid biopsies;
- ▶ to provide evidence of activity of metformin plus chloroquine related to *D*-2HG levels in the serum, urine, bile and/or tumoural mass of patients with *IDH1/2*-mutated chondrosarcoma, glioma or intrahepatic cholangiocarcinoma.

Trial end points

Primary end points (outcomes)

- ▶ We will determine the MTD, which is the chloroquine plus metformin dose in which ≤ 1 in three patients (of a 3+3 dose-escalation schedule) shows serious adverse effects.
- ▶ We will determine the RD of chloroquine plus metformin, which is the dose level one step below the MTD.

Secondary end points (outcomes)

- ▶ Serum metformin and chloroquine concentrations will be measured to investigate the pharmacokinetics of this combination and establish a relationship or not between drug exposure and toxicity and/or efficacy.
- ▶ Tumour size will be measured using a MRI and/or CT scan before and after treatment with metformin plus chloroquine to monitor tumour response

using response evaluation criteria in solid tumours (RECIST) 1.1 in patients with chondrosarcoma and intrahepatic cholangiocarcinoma and response assessment in neuro-oncology (RANO) in patients with glioma.

- ▶ *D*-2HG concentrations in serum, urine, bile and/or the tumoural mass will be measured by MS every 4 weeks during treatment and by MRS at the start and end of the treatment to investigate the effects of metformin plus chloroquine on *D*-2HG levels. Furthermore, these *D*-2HG measurements will be compared with results obtained from CT and/or MRI scans to investigate whether determinations of *D*-2HG concentrations in serum, urine, bile and/or the tumoural mass correlate with radiologically observed tumour responses to therapy.
- ▶ The variant allelic frequency of *IDH1* mutations or *IDH2* mutations will be measured using NGS on liquid biopsies at the start and end of the treatment and every 4 weeks during treatment to determine the effects of metformin plus chloroquine on the variant allelic frequency and mutational load of these mutations.

Participants

In brief, this trial will enrol eligible patients with *IDH1/2*-mutated and newly diagnosed, recurrent, relapsed or refractory and/or metastasised WHO grade II–III chondrosarcoma,⁶³ WHO grade II–IV glioma⁶⁴ or intrahepatic cholangiocarcinoma. All inclusion and exclusion criteria are listed in box 1. The trial will enrol patients who have no tumour resection planned (figure 2) and those who have a tumour (re-)resection planned (figure 3). These patients will be studied in their waiting period until resection (approximately 6–8 weeks). We are especially interested in patients who had a tumour resection in the past of which tumour material is available, who had a recurrence of their tumour and who will have a re-resection of this recurrent tumour, because we will then be able to collect pretreatment and post-treatment samples of these patients. This may also be achieved using sequential tumour biopsies. For patients who have no tumour resection planned, the end of the study is defined as when a patient chooses to withdraw from the study, when a patient experiences a dose-limiting toxicity (DLT) or when tumour progression occurs. For patients who will have a tumour resection, the study will be conducted during the waiting period until surgery. The end of study is defined similarly as for patients who have no tumour resection planned or 2 days before surgery.

This phase Ib/II dose-finding study has three dose-escalation levels. According to a 3+3 dose-escalation scheme, we need a maximum of 18 patients (a maximum of six patients in three dose-escalation levels). A maximum of 10 patients can be enrolled of each tumour type (chondrosarcoma, glioma or intrahepatic cholangiocarcinoma).

Box 1 Inclusion and exclusion criteria

Key inclusion criteria

- ▶ Age ≥ 18 years.
- ▶ Presence of a measurable intrahepatic cholangiocarcinoma or WHO grade II–III chondrosarcoma (RECIST 1.1 criteria⁶⁵) or WHO grade II–IV glioma (RANO criteria⁶⁶), both newly diagnosed and refractory, relapsed or recurrent tumours.
- ▶ Tumour carries a *D*-2HG-generating mutation in *IDH1* or *IDH2* as determined by sequencing of primary tumour DNA, immunohistochemistry of primary tumour tissue with an IDH1/2 mutant-specific antibody, or MRS imaging of the tumour (for patients with glioma).
- ▶ Eastern Cooperative Oncology Group (ECOG)/WHO performance status 0–2.
- ▶ Adequate renal function (creatinine <150 $\mu\text{mol/L}$ or a creatinine clearance >60 mL/L).
- ▶ Adequate liver function (bilirubin <1.5 times the normal upper limit; Alanine transaminase (ALT) and Aspartate transaminase (ASAT) <2.5 the normal upper limit).
- ▶ Adequate bone marrow function (white blood cells $>3.0 \times 10^9/\text{L}$, platelets $>100 \times 10^9/\text{L}$).
- ▶ When patient is eligible for tumour resection, surgery is planned at least 4 weeks later than the start of study treatment.

Key exclusion criteria

- ▶ Concomitant other anticancer therapy (eg, surgical resection, chemotherapy, targeted therapy, radiation therapy, surgery). Palliative therapy is permitted, such as:
 - ▶ palliative radiotherapy for symptomatic bone metastases,
 - ▶ dexamethasone for symptom relief in patients with glioma and cerebral oedema,
 - ▶ non-enzyme inducing antiepileptic drugs (with the exception of topiramate) in patients with glioma and epileptic seizures.
- ▶ Severe and/or uncontrolled medical conditions at <6 months prior to randomisation, such as:
 - ▶ unstable angina pectoris, symptomatic congestive heart failure, myocardial infarction or cardiac arrhythmias,
 - ▶ pulmonary insufficiency,
 - ▶ severe gastrointestinal, neurological (including epilepsy) or haematological diseases (interaction with chloroquine),
 - ▶ uncontrolled diabetes as defined by fasting serum glucose >12 mmol/L ,
 - ▶ active or uncontrolled severe infection, including malaria,
 - ▶ cirrhosis, chronic active hepatitis or chronic persistent hepatitis.
- ▶ Serious concomitant systemic disorder that compromises the safety of the patient, at the discretion of the investigator.
- ▶ Patients who have a known history of alcohol abuse (interaction with metformin).
- ▶ Patients with known glucose-6-phosphate dehydrogenase deficiency, porphyria, myasthenia gravis or ocular/retinal aberrations (interactions with chloroquine).
- ▶ Patients who use digoxin, monoamine oxidase (MAO) inhibitors, phenylbutazone, oxyphenbutazone, gold preparations or cimetidine (known pharmacokinetic interactions with chloroquine), or loop diuretics (known pharmacokinetic interaction with metformin) for which not a good alternative is available.
- ▶ Patients with a known hypersensitivity to metformin or chloroquine.
- ▶ Use of metformin or chloroquine in the previous 6 months or long-term use of chloroquine (>5 years or cumulative dose >300 g) in the past.

ctDNA, circulating tumour DNA; *D*-2HG, *D*-2-hydroxyglutarate; IDH1/2, isocitrate dehydrogenase 1 and 2; MRS, magnetic resonance spectroscopy; MS, mass spectrometry; RANO, response assessment in neuro-oncology; RECIST, response evaluation criteria in solid tumours.

Figure 2 Dosing schedule and study design for patients who will not undergo tumour resection. *D*-2HG, *D*-2-hydroxyglutarate; IHC, immunohistochemistry; MRS, magnetic resonance spectroscopy; MS, mass spectrometry; NGS, next-generation sequencing; q.d., once a day.

Figure 3 Dosing schedule and study design for patients who have a tumour resection planned. Surgery will define the end of this time schedule (2 days before surgery). D-2HG, D-2-hydroxyglutarate; IHC, immunohistochemistry; MRS, magnetic resonance spectroscopy; MS, mass spectrometry; NGS, next-generation sequencing; q.d., once a day.

Dose of study drugs and dose-escalation schedule

Metformin

The starting dose of metformin will be 500 mg by mouth once a day during the first 5 days. Subsequently, the metformin dose will be escalated as outlined in table 1. This escalation schedule is based on an earlier phase II clinical trial in pancreatic adenocarcinoma.⁶⁷ The purpose of the lower metformin starting dose is to reduce side effects of metformin, especially gastrointestinal side effects. This starting dose mimics dosage schedules of metformin treatment in patients with type 2 diabetes mellitus.

Chloroquine

Chloroquine will be added to metformin in week 2 of the study and chloroquine doses will not be escalated. Patients who have no tumour resection planned will be treated with 200 mg chloroquine once a day. For patients who have a tumour resection planned, chloroquine will be given in a step-down dosing schedule. The starting dose (first 2 weeks of chloroquine administration; weeks 2 and 3 of study) is 300 mg once a day. In subsequent weeks (week 4 of the study and later), the chloroquine maintenance dose will be 200 mg once a day. Because we expect the study duration to be a few weeks in patients with resectable tumours (there usually is a waiting time of 6–8 weeks from diagnosis until surgery), the higher starting dose in patients with resectable tumours allows

build-up of functional chloroquine serum concentrations in a shorter time, thereby increasing the chance of a measurable effect within the period of time in which the study will be conducted. This dosing schedule is necessary because of the long half-life of chloroquine. Step-down dosing schedules of chloroquine are also used in systematic lupus erythematoses.⁶⁸

Dose finding

The rate of subject entry and escalation to the next dose level will depend upon assessment of the safety profile of patients entered at the previous dose level. Toxicity will be evaluated according to the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.03. A minimum of three patients will be entered on each dose level. Online supplementary file 1 describes the standard 3+3 dose-escalation schedule of our study, the procedures for inpatient dose escalation and patient replacement, and the finding of the recommended phase II dose. Dose (de)escalation will be based on the toxicity assessment in the first 8 weeks of therapy and the documentation of any DLTs. To be considered as a DLT, the toxicity must be considered to be related to the study drug. DLTs are defined in online supplementary table 1. When a patient experiences a DLT, he/she can decide to withdraw from the study or go into inpatient de-escalation by receiving metformin at one dose level lower than the dose level that provoked the DLT.

Screening for IDH1/2 mutations

The IDH1/2 mutational status of patients will be assessed using DNA sequencing or immunohistochemistry. In a patient with glioma, the presence of an IDH1/2 mutation can also be established using MRS to detect intratumoural 2HG levels.^{54 55}

Study visits

Patients with IDH1/2-mutated tumours will visit their hospital of inclusion once for additional eligibility screening (see inclusion and exclusion criteria). Once enrolled in the study, patients will undergo a study visit after 1 week, in which blood will be drawn for

Table 1 Metformin dose-escalation schedule

Dose level	Dose of metformin given orally (total daily dose)	Minimum number of patients
-1	500 mg once a day (500 mg total)	—
1 (starting)	500 mg two times a day (1000 mg total)	3
2	1000 mg two times a day (2000 mg total)	3
3	1500 mg two times a day (3000 mg total)	3

Table 2 Timeline, study treatment, study visits and medical procedures

Required investigations	Screening	Day 8 (week 2)	Day 29/week 5 and every 4 weeks thereafter	Day 57/week 9 and every 8 weeks thereafter	End of study
Visit number	1	2	3	4+	4+
Written informed consent	Prior to screening				
Demographics (age, sex)	X				
Overall medical history	X				X
Physical examination, including weight and height	X		X	X	X
Vital signs (blood pressure, pulse)	X		X	X	X
ECOG/WHO performance status	X		X	X	X
CT or MRI scan of measurable lesion, ≤1 month prior to start treatment	X			X	X
Haematology	X		X	X	X
Serum chemistry					
Hepatic function	X		X	X	X
Renal function	X		X	X	X
Glucose	X		X	X	X
HbA1c	X			X	X
Triglycerides	X			X	X
Cholesterol	X			X	X
Haemostatic parameters (aPTT and PT)	X				X
Insulin, IGF-1, IGF-binding protein 3	X			X	X
Vitamin B ₁₂	X			X	X
Metformin concentration		X	X	X	X
Chloroquine concentration			X	X	X
MS of serum/urine/bile for D-2HG levels	X		X	X	X
MRS for intratumoural 2HG levels	X				X
Liquid biopsy	X		X	X	X
ECG	X				
Pregnancy test	X				
Optional: tumour biopsy	X			X	X

In addition to this scheme, an ECG will be performed every 24 weeks. Metformin and chloroquine concentrations will be taken at the end of study only when possible.

aPTT, activated partial thromboplastin time; (D-)2HG, (D-)2-hydroxyglutarate; ECOG, Eastern Cooperative Oncology Group; IGF, insulin growth factor; MRS, magnetic resonance spectroscopy; MS, mass spectroscopy; PT, prothrombin time.

pharmacokinetic analysis (see below) and after 4 weeks, in which blood will be drawn for serum D-2HG MS analysis, for analysis of haematologic, hepatic, renal, and chemistry parameters and for further pharmacokinetic analyses. Every 8 weeks, these patients will have a more elaborate study visit in which they will undergo a CT/MRI scan in addition to the procedures that will also occur at study visits every 4 weeks. Specifics for each study visit are shown in [table 2](#).

Pharmacokinetics

Pharmacokinetics of metformin and chloroquine are monitored in order to evaluate a relationship between

drug exposure, toxicity and/or efficacy. Furthermore, the magnitude of the pharmacokinetic interactions between both compounds will be assessed. Blood samples will be taken at several time points during the study for the determination of the respective plasma levels.

The half-life of metformin is ± 6.5 hours,⁶⁹ which means that with daily dosing the plasma level of metformin reaches a steady-state concentration within 2 days. The half-life of chloroquine is considerably longer (± 2 weeks),⁷⁰ which means that with daily dosing the plasma level of chloroquine reaches a steady-state concentration within 8 weeks in a flat-dosed scheme (which applies to

patients who will have no tumour resection) and ± 4 to 6 weeks under the proposed step-down dose scheme (see above).

Predose plasma samples (ie, prior to study medication ingestion) will be taken on day 8 (week 2), day 29 (week 5) and every 4 weeks thereafter (see table 2). Because chloroquine administration starts on day 8, the predose plasma sample on that day contains a metformin plasma concentration that reflects metformin monotherapy. The pharmacokinetic interaction between metformin and chloroquine is evaluated by comparing the metformin concentration on day 8 with the metformin concentration at subsequent time points. The relationship between exposure and toxicity is evaluated using all samples. The difference in the time after which steady-state serum levels of metformin and chloroquine are reached also helps with distinguishing the source of any drug-related toxicity, because any toxicity in the first month is unlikely to be the result of chloroquine, but likely the result of metformin.

Detection of D-2HG levels in serum, urine and/or bile

We will detect D-2HG levels in patient serum, urine and/or bile using MS. Because our method distinguishes the *IDH1/2* mutation-specific D-2HG from the unspecific L-2HG, we expect a better signal-to-noise ratio and a higher sensitivity and specificity to detect *IDH1/2* mutations than in previous studies, where total 2HG levels were measured.^{52 53 57} Bile samples will only be obtained from patients with intrahepatic cholangiocarcinoma with easy access to bile samples in the context of regular patient care, such as a percutaneous transhepatic biliary drain.

Detection of intratumoural 2HG levels

Intratumoural 2HG levels will be detected using long-echo MRS point-resolved spectroscopy (PRESS) on a 3T MRI at the start and end of treatment of patients using protocols that were described before.⁵⁵ We will compare intratumoural 2HG levels before and after treatment to investigate whether MRS can be used to monitor therapy responses in *IDH1/2*-mutated solid tumours. We will also compare results from MRS with the results of DNA sequencing or immunohistochemistry to investigate whether MRS can be used to determine the mutational status of *IDH1/2* in patients with chondrosarcoma or intrahepatic cholangiocarcinoma.

Therapy response assessment

Response will be assessed by RECIST 1.1 guidelines⁶⁵ for chondrosarcoma and intrahepatic cholangiocarcinoma or RANO guidelines⁶⁶ for glioma on images obtained with CT and/or MRI scans. Scans will be performed at screening and every 8 weeks from study inclusion. We will investigate whether NGS and MS analysis of ctDNA and plasma fractions, respectively, derived from blood samples that will be taken before, during and after the study treatment, can be used to monitor therapy responses. When there is prestudy and poststudy primary tumour material

available we will perform immunohistochemical staining with the appropriate *IDH1/2* mutant-specific antibody to investigate the intratumoural mutational burden.

Toxicity monitoring

Patients will be interviewed for toxicity every 4 weeks and will be educated on frequently occurring side effects of chloroquine and metformin (gastrointestinal side effects, signs of hypoglycaemia). Prolongation of corrected QT interval (QTc) time is a rare adverse effect of chloroquine and patients will undergo an ECG every 24 weeks. Large cumulative doses (>460 g) of chloroquine can induce retinopathy (Bull's eye maculopathy).⁷¹ Daily doses of up to 250 mg per day for several years are considered to carry an acceptable risk for chloroquine-induced retinopathies.⁷² In the proposed clinical trial, patients will be treated with 200 mg chloroquine per day (cumulative dose per year: 73 g). Therefore, this clinical trial carries a very low risk to induce chloroquine-related retinopathies. Long-term use of chloroquine (>5 years or >300 g cumulative dose) is an exclusion criterion for this trial to prevent chloroquine-related retinopathies. We will perform an ophthalmologic evaluation when the estimated lifetime chloroquine dose of a patient exceeds 300 g during his/her trial participation.

Statistical methods

The patient sample size in the clinical trial (n=20) is based on the 3+3 dose-escalation schedule and the three proposed dose-escalation steps. With 20 patients, we are able to determine whether dose level 3 is the MTD, even when we need a 3+3 expansion cohort at step 2 and step 3 and when 25% of patients are not evaluable because the patients discontinued their study participation before completing 4 weeks of study treatment. Tumour volumes (from CT/MRI scans), serum metformin, chloroquine and D-2HG concentrations (from MRS/MS measurements), and *IDH1/2* mutational loads (from NGS) from before, during and after treatment time points will be compared using the paired samples *t*-test.

Data management, auditing and access

Source data from the trial will be locally stored and entered in electronic case report forms. Based on the guidelines by the NFU (Dutch Federation of University Medical Centers), the risk of this study was qualified as 'moderate.' According to this, a 'minimal intensive auditing' is advised, which will be performed by an independent clinical research associate (for details, see online supplementary file 1). Besides this clinical research associate, only the investigators are allowed access to the source data. As part of informed consent, patients will be informed as to the strict confidentiality of their patient data, but that their medical records may be reviewed for trial purposes by clinical research associates. Being primarily a phase Ib dose-finding study, this clinical trial does not have a data and safety monitoring board.

Informed consent

All patients will be informed by the investigator(s) of the aims of the study, the possible adverse events, the procedures, the possible hazards to which he/she will be exposed, who has access to their patient data and what provisions were made for compensating those who suffer harm from trial participation. It will be emphasised that the participation is voluntary and that the patient is allowed to refuse further participation in the protocol whenever he/she wants. This will not prejudice the patient's subsequent care. Documented informed consent must be obtained for all patients included in the study before they are enrolled in the study.

Harms

(Serious) adverse events and (serious) adverse drug reactions will be collected and recorded throughout the study period, starting at day 1 of the treatment through 1 month after the last dose of investigational product in accordance with Good Clinical Practice guidelines as described in the International Conference on Harmonisation Guideline (ICH-GCP). It will be left to the investigator's clinical judgement to determine whether an adverse event is related and of sufficient severity to require the subject's removal from treatment or from the study. A subject may also voluntarily withdraw from treatment. A potential harm for patients concerns overlapping side effects of metformin and chloroquine, which are mainly of gastrointestinal nature.

ETHICS AND DISSEMINATION

This study is being conducted according to Good Clinical Practice guidelines as described in the International Conference on Harmonisation Guideline (ICH-GCP) and in accordance with general ethical principles outlined in the Declaration of Helsinki. Ethical approval was obtained from the Medical-Ethical Committee of the Academic Medical Centre, Amsterdam (MEC-AMC), the Netherlands, and the Dutch national competent authority (Centrale Commissie Mensgebonden Onderzoek (CCMO)) on 22 October 2015 and 13 January 2016, respectively, under reference number NL53150.018.15. Informed consent forms were approved by the MEC-AMC.

A report describing the results of the study will be submitted to a peer-reviewed journal. Where permitted by patient data protection standards, data will be published and shared together with the publication of the study results. Coauthorship will be based on standard International Committee of Medical Journal Editors (ICMJE) guidelines. No professional writers will be used.

An ethical limitation of the present clinical trial may be that the therapeutic index of metformin and chloroquine has been established in glioma and colorectal carcinoma cells,¹⁶ but not in intrahepatic cholangiocarcinoma or chondrosarcoma models. However, this is primarily a dose-finding study. Follow-up phase II clinical trials will be rationally designed based on the pending evidence

whether or not the efficacy of metformin and chloroquine treatment will be validated in model systems of other types of cancer by then.

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Competing interests None declared.

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