Towards personalised medicine for cancer

From initial therapy to follow-up

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Chapter 8: Study protocol of a phase Ib/II clinical trial of metformin and chloroquine in patients with IDH1-mutated or IDH2-mutated solid tumours


Abstract
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, emphasizing the need for novel therapies. Mutations in the genes IDH1 and IDH2 (isocitrate dehydrogenase 1 and 2) occur in 60% of chondrosarcoma, 80-90% of WHO grade II-IV glioma and 20% of intrahepatic cholangiocarcinoma. IDH1/2-mutated cancer cells produce the oncometabolite D-2-hydroxyglutarate (D2HG) and are metabolically vulnerable to treatment with the oral antidiabetic metformin and the oral antimalarial drug chloroquine. 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 or not tumour responses can be measured by non-invasive D2HG measurements (mass spectrometry [MS] and magnetic resonance spectroscopy [MRS]) 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.

Introduction
IDH1 and IDH2 are homodimeric enzymes that reversibly convert isocitrate to α-ketoglutarate (αKG) in cytoplasm and mitochondria, respectively. Somatic heterozygous mutations in IDH1/2 that produce the oncometabolite D2HG are observed in substantial percentages of various tumour types such as chondrosarcoma (60%), WHO grade II-III glioma (80-90%), secondary WHO grade IV glioblastoma (80%), and intrahepatic cholangiocarcinoma (20%). 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%). In chondrosarcoma and glioma, IDH1/2 mutations are considered very early or even inaugural genetic defects, and are thus present in a large fraction of, or even all, cancer cells. This renders IDH1/2 mutations an interesting target for anti-cancer treatment because such tumour homogeneity decreases the risk of therapy resistance. Recently, inhibitors of mutant IDH1 and IDH2 were developed that may be effective in stalling malignant progression of early-stage IDH1/2-mutated cancers.

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 are considered to be highly therapy resistant. Consequently, the 1-year survival rate of metastasised high-grade chondrosarcoma is <10%. Gliomas vary from WHO grade II diffuse astrocytoma and diffuse oligodendroglioma, with median survivals of more than
five years, to WHO grade IV glioblastoma, with a median survival of only 15 months despite aggressive treatment using radiotherapy and temozolomide. Gliomas are diffusely growing tumours, which renders surgery ineffective, emphasizing the dire need for novel therapies. Furthermore, the blood-brain barrier (BBB) prohibits the use of most chemotherapeutics and the surrounding normal brain limits aggressive radiotherapy regimens due to limitations that are raised by healthy brain tissue. Intrahepatic cholangiocarcinoma is resectable in only 40% of patients. In unresectable cases, intrahepatic cholangiocarcinoma patients 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.

**Metabolic effects of IDH1/2 mutations.** Heterozygous hotspot IDH1/2 mutations disable IDH1/2 wild-type enzyme activity and induce a neo-enzymatic activity that leads to the production and subsequent accumulation of D2HG. D2HG is normally present only in trace amounts in normal tissues and cells but accumulates up to 50 mM in IDH1/2-mutated glioma. D2HG is chemically very similar to αKG and may inhibit over 60 αKG-dependent enzymes, resulting in global DNA/histone hypermethylation, decreased hypoxia-inducible factor 1a (HIF1α) expression, and perturbed collagen maturation. Depending on the cellular context, these effects are the basis of oncogenesis and imply a dependence on D2HG of early-stage IDH1/2-mutated tumours.

IDH1/2-mutated cancer cells need αKG to synthesise D2HG and fuel the tricarboxylic acid (TCA) cycle to support their metabolism. αKG is generated by glycolysis (glucose breakdown) or glutaminolysis (glutamine/glutamate breakdown). IDH1/2 mutations downregulate αKG levels by consuming αKG and by inhibition of αKG production via direct effects, i.e. by disabling IDH1/2 wild-type kinetics, and indirect effects, e.g. by decreasing TCA cycle activity. Therefore, IDH1/2-mutated cancer cells rely on glutaminolysis for sufficient αKG supply to generate the oncometabolite D2HG (Figure 1). The conversion of glutamate to αKG is catalysed by glutamate dehydrogenase (GDH), which is the final step of glutaminolysis and can be inhibited by the anti-malaria drug chloroquine and the antidiabetic drug metformin. In addition, IDH1-mutated glioma cells show increased levels of autophagy, likely as a survival mechanism of cells to metabolic stress by catabolizing proteins in order to provide substrates for energy production in stress/starvation contexts. Autophagy is inhibited by chloroquine and the anti-cancer 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 D2HG. More specifically, D2HG inhibits enzymatic activity of complex IV (cytochrome C oxidase) of the ETC and the TCA(-like) enzymes IDH1/2 and αKG dehydrogenase. This reduces oxidative phosphorylation, the primary source of ATP in cancer cells. 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 biguanide metformin and phenformin, which selectively restrict the proliferation of these cells. Another metabolic vulnerability of IDH1/2-mutated glioma may be their excess deposition of the acidic D2HG in their microenvironment, which is hypothesised to contribute to their diffuse growth. Chloroquine buffers the tumour milieu 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 while in chondrosarcoma a correlation between mutation and survival was absent. 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. Combined, these data suggest that at least some types of cancer with IDH1/2 mutations should be targeted by compounds that exploit this presumed
Metformin and chloroquine for IDH1/2-mutated solid tumors

metabolic vulnerability rather than compounds that decrease metabolic stress (i.e. 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 by IDH1/2 mutations against anti-cancer therapy.\textsuperscript{15,143,212} 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.\textsuperscript{255,291} 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-approved for other indications. The safety profiles of metformin and chloroquine are favourable over other anti-cancer 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 anti-cancer research efforts. The potential of metformin and chloroquine as adjuvant drugs was recently demonstrated \textit{in vivo}, where metformin or chloroquine had a sensitizing and/or synergistic anti-tumour effect in combination with temozolomide,\textsuperscript{292,293} cisplatin,\textsuperscript{294,295} and gemcitabine\textsuperscript{296,297} 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 human cancer to ionizing radiation.\textsuperscript{298,299} 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 Encyclopedia\textsuperscript{300} and see Chapter 7). 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

![Figure 1. Cellular carbohydrate and glutamine metabolism showing the pathways in which wild-type IDH1 and IDH2 are functional.](image-url)

\textit{Isocitrate dehydrogenase 1, 2, and 3 (IDH1-3) catalyse the conversion of isocitrate to α-ketoglutarate (αKG) 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 αKG 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\textsuperscript{WT}) differs from mutant IDH1/2 (IDH1/2\textsuperscript{MUT}) because the latter enzyme converts αKG into a novel oncometabolite, D-2-hydroxyglutarate (D2HG).}
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 anti-tumour 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 1970s 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. 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 destruct the BBB, in vivo experiments in mice have shown that metformin and chloroquine adequately pass the blood-brain barrier.

### Non-invasive detection of IDH1/2 mutations

The gold standard of IDH1/2 mutation detection is mutational analysis of tumour DNA. However, in glioma 90% of all IDH1/2 mutations are IDH1R132H and its presence can be reliably detected using an immunohistochemistry of glioma tissue with an IDH1R132H-specific antibody. The presence of IDH1/2 mutations in AML and intrahepatic cholangiocarcinoma can be easily, reliably, and non-invasively detected via determination of 2HG levels or D2HG levels in serum or urine by mass spectrometry (MS). Furthermore, MS-determined 2HG serum levels correlate with therapy response in these cancers. 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%. 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. Conversely, serum 2HG levels correlate poorly with the IDH1/2 mutational status in glioma due to a limited blood-brain barrier passage of D2HG. Urine 2HG levels are higher in patients with IDH1-mutated glioma than in patients with IDH1 wild-type glioma, 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. Most aforementioned measurements determined total 2HG levels and thus did not discriminate between the D-enantiomer of 2HG (which is specific for IDH1/2 mutants) and the L-enantiomer of 2HG (which is unspecific and is generated during hypoxia). Better separation of D2HG and L2HG may allow for IDH1/2 mutational status predictions with higher sensitivity and specificity. Besides methods that detect D2HG 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.

### 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. 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 anti-cancer 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 D2HG 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 exists, or besides conventional anti-cancer treatments such as radiation and temozolomide in glioma and cisplatin and gemcitabine in intrahepatic cholangiocarcinoma.

Methods and analysis

Overall study design. MACIST (Metformin and chloroquine in IDH1/2-mutated solid tumours) is a nonrandomised, open-label, dose-finding, multi-centre 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).

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 D2HG and L2HG 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 chondrosarcoma and intrahepatic cholangiocarcinoma patients 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 D2HG 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) show 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 chondrosarcoma and intrahepatic cholangiocarcinoma patients and response assessment in neuro-oncology (RANO) in glioma patients.

- $D_2HG$ concentrations in serum, urine, bile, and/or the tumoural mass will be measured by MS every four 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 four 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 metastatised WHO grade II-III chondrosarcoma, WHO grade II-IV glioma, or intrahepatic cholangiocarcinoma. All inclusion and exclusion criteria are listed in Table 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 that 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 pre- and post-treatment samples of these patients. This may also be achieved using sequential tumour biopsies. For patients that 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 that have no tumour resection planned or two 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 6 patients in 3 dose escalation levels). A maximum of 10 patients can be enrolled of each tumour type (chondrosarcoma, glioma, or intrahepatic cholangiocarcinoma).

**Dose of study drugs and dose escalation schedule**

**Metformin.** The starting dose of metformin will be 500 mg per os q.d. during the first five days. Subsequently, the metformin dose will be escalated as outlined in Table 2. This escalation schedule is based on an earlier phase II clinical trial in pancreatic adenocarcinoma. The purpose of the lower metformin starting dose is to reduce side effects of metformin, especially gastro-intestinal 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 q.d. For patients who have a tumour resection planned, chloroquine will be given in a step-down dosing schedule. The starting dose (first two weeks of chloroquine administration; week
Table 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 criteria \(^{317}\)) or WHO grade II-IV glioma (RANO criteria \(^{318}\)), both newly-diagnosed and refractory, relapsed, or recurrent tumours.
- Tumour carries a D2HG-generating mutation in \(\text{IDH1}\) or \(\text{IDH2}\) as determined by sequencing of primary tumour DNA, immunohistochemistry of primary tumour tissue with an \(\text{IDH1/2}\) mutant-specific antibody, or MRS imaging of the tumour (for glioma patients).
- ECOG/WHO performance status 0-2.
- Adequate renal function (creatinine <150 μmol/L or a creatinine clearance >60 ml/L).
- Adequate liver function (bilirubin <1.5 times the normal upper limit; ALAT and ASAT <2.5 the normal upper limit).
- Adequate bone marrow function (white blood cells >3.0 x 10⁹/L, platelets >100 x 10⁹/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 anti-cancer therapy (e.g. 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 or cerebral oedema,
  - non-enzyme inducing anti-epileptic drugs (except topiramate) in patients with glioma or epilepsy.
- 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, MAO inhibitors, fentanylbutazone, oxygenbutazone, 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 grams) in the past.

Abbreviations: ctDNA, circulating tumour DNA; D2HG, D-2-hydroxyglutarate; IDH1/2, isocitrate dehydrogenase 1 and 2; MRS, magnetic resonance spectroscopy; MS, mass spectrometry.

2 and 3 of study) is 300 mg q.d. In subsequent weeks (week 4 of the study and later), the chloroquine maintenance dose will be 200 mg q.d. 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.\(^{319}\)
**Table 2. Metformin dose escalation schedule.**

<table>
<thead>
<tr>
<th>Dose level</th>
<th>Dose of metformin given orally (total daily dose)</th>
<th>Minimum number of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>-1</td>
<td>500 mg q.d. (500 mg total)</td>
<td>-</td>
</tr>
<tr>
<td>1 (starting)</td>
<td>500 mg b.i.d. (1000 mg total)</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>1000 mg b.i.d. (2000 mg total)</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>1500 mg b.i.d. (3000 mg total)</td>
<td>3</td>
</tr>
</tbody>
</table>

**Abbreviations:** b.i.d., two times a day; q.d., once a day.

**Dose finding.** The rate of subject entry and escalation to the next dose level will depend upon assessment of the safety profile of patients 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 3 patients will be entered on each dose level. Dose (de)escalation will be based on the toxicity assessment in the first eight weeks of therapy and the occurrence of DLTs (Table 3). To be considered as a DLT, the toxicity must be considered to be related to the study drug. When a patient experiences a DLT, he/she can decide to withdraw from the study or go into intrapatient de-escalation by receiving metformin at one dose level lower than the dose level that provoked the DLT.

**3+3 dose-escalation schedule and intrapatient dose-escalation**

**Maximum administered dose.**

- If 0/3 patients exhibit dose-limiting toxicity at this dose level, dose escalation to the next dose level may begin in a new cohort of patients. Patients enrolled on the previous dose level who are still receiving therapy may now undergo intrapatient dose escalation to this new dose level provided that they have experienced no drug related toxicity of grade 2 or higher at the previous dose level.
- If 1/3 patients exhibit dose-limiting toxicity at this dose level, expand dose level to a total of six patients. Toxicity information from patients who underwent intrapatient dose escalation can be used for expansion cohorts, but only when they have completed at least 8 weeks of treatment at the new dose level.
  - If no further DLT events are observed, dose escalation to the next dose level may begin in a new cohort of patients and patients enrolled on the previous dose level who are still receiving therapy may now undergo intrapatient dose escalation to this dose level provided that they have experienced no drug related toxicity of grade 2 or higher.
  - If further DLTs are observed (i.e. in ≥2/6 patients), this dose level will be considered the maximum administered dose (MAD).
- If ≥2/3 patients exhibit dose-limiting toxicity, this dose level will be considered the MAD. If this toxicity occurs at level 1 (starting level), dose de-escalation to level -1 will be applied.

**Recommended phase II dose.** As described in the full text manuscript, the MAD is the dose in which ≥2/3 or ≥2/6 patients experience a DLT, or the final dose from the dose escalation schedule (1500 mg metformin b.i.d. and 200 mg chloroquine q.d.). One dose level below the MAD will be considered the RD for follow-up phase II clinical trials. When the starting dose level (“1”) is the MAD, we will de-escalate the dose level to dose level “-1”. When we do not observe DLTs in three patients or one DLT in six patients at this dose level, then dose level “-1” will be the RD. When we observe more than one DLT, the combination of metformin and chloroquine will be considered too toxic to be useful in cancer patients. In contrast to this situation where we have to accept the lowest dose-escalation level as the RD, when 0/6 patients experience DLTs at the final dose level of the dose escalation schedule (i.e. dose level “3”), this can be considered the RD for follow-up phase II clinical trials, instead of dose level “2”. Up to a total of six patients may be treated at the RD level to assure information on the safety profile when that dose is complete. When clinically appropriate, intermediate dose levels may be
studied to assure that the RD is the highest tolerable. Furthermore, when pharmacokinetic data suggests that saturating absorption of drug is occurring on a b.i.d. oral administration level, further dose splitting to three times a day or four times a day schedules may be considered.

**Patient replacement.** Three patients within a dose level must be observed for eight weeks before accrual to the next dose level may begin. If a patient is withdrawn from the study prior to completing 22 days of therapy without experiencing a DLT prior to withdrawal, an additional patient may be added to that dose level. Patients missing seven or more doses (one week) due to toxicity will not be replaced since these patients will be considered to have experienced a dose-limiting toxicity.

**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.84,86

**Study visits.** Patients with IDH1/2-mutated tumours will visit their hospital of inclusion once for additional eligibility screening (see in- and exclusion criteria). Once enrolled in the study, patients will undergo a study visit after one week, in which blood will be drawn for pharmacokinetic analysis (see below) and after four weeks, in which blood will be drawn for serum D2HG MS analysis, for analysis of haematologic, hepatic, renal, and chemistry parameters and for further pharmacokinetic analyses. Every eight 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 four weeks. Specifics for each study visit are shown in Table 4.

**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,320 which means that with daily dosing the plasma level of metformin reaches a steady-state concentration within two days. The half-life of chloroquine is considerably longer (±2 weeks),321 which means that with daily dosing the plasma level of
Table 3. Haematologic and non-haematologic dose-limiting toxicities (DLT).

<table>
<thead>
<tr>
<th>Haematologic</th>
<th>Non-haematologic</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Absolute granulocyte count &lt;0.5 x 10^9/l.</td>
<td>• Diarrhoea &gt; grade 3 despite optimal loperamide use.</td>
</tr>
<tr>
<td>• Febrile neutropenia (ANC &lt;1.0 x 10^9/L, fever &gt;38.5°C).</td>
<td>• Rash &gt; grade 3 or grade 2 is medically concerning or unacceptable to the patient.</td>
</tr>
<tr>
<td>• Platelets &lt;25 x 10^9/l.</td>
<td>• Other grade 3 effects considered to be treatment related.</td>
</tr>
<tr>
<td>• Bleeding due to thrombocytopenia, as determined by a physician.</td>
<td>• Missing &gt;7 days of treatment for toxicity reasons.</td>
</tr>
</tbody>
</table>

Grading of side effects is performed using CTCAE. Abbreviations: ANC, absolute neutrophil count.

chloroquine reaches a steady-state concentration within eight weeks in a flat-dosed scheme (which applies to patients who will have no tumour resection) and ±4-6 weeks under the proposed step-down dose scheme (see above). Predose plasma samples (i.e. prior to study medication ingestion) will be taken on day 8 (week 2), day 29 (week 5) and every four weeks thereafter (see Table 4). 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 help 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 D2HG levels in serum, urine, and/or bile. We will detect D2HG levels in patient serum, urine, and/or bile using MS. Because our method distinguishes the IDH1/2 mutation-specific D2HG from the unspecific L2HG, 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.83,306,308 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 (PRESS) on a 3T MRI at the start and end of treatment of patients using protocols that were described before.86 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 guidelines317 for chondrosarcoma and intrahepatic cholangiocarcinoma or RANO guidelines318 for glioma on images obtained with CT and/or MRI scans. Scans will be performed at screening and every eight 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 pre-study and post-study 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 educated on frequently occurring side-effects of chloroquine and metformin (gastro-intestinal side-effects, signs
Table 4. Timeline, study treatment, study visits and medical procedures.

<table>
<thead>
<tr>
<th>Required investigations</th>
<th>Screening</th>
<th>Day 8 (week 2)</th>
<th>Day 29/week 5 and every 4 weeks thereafter</th>
<th>Day 57/week 9 and every 8 weeks thereafter</th>
<th>End of study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visit number</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4+</td>
<td>4+</td>
</tr>
<tr>
<td>Written informed consent</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Demographics (age, sex)</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall medical history</td>
<td>X</td>
<td></td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Physical examination, including weight and height</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Vital signs (blood pressure, pulse)</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>ECOG/WHO performance status</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>CT or MRI scan of measurable lesion, ≤1 month prior to start treatment</td>
<td>X</td>
<td>X</td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Haematology</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Serum chemistry:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatic function</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Renal function</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>HbA1c</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Triglycerides</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Cholesterol</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Haemostatic parameters (aPPT and PT)</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Insulin, IGF-1, IGF binding protein-3</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Vitamin B12</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Metformin concentration</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Chloroquine concentration</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>MS of serum/urine/bile for D2HG levels</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>MRS for intratumoural 2HG levels</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Liquid biopsy</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>ECG</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Pregnancy test</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Optional: tumour biopsy</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
</tbody>
</table>

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. Abbreviations: (D-)2HG, (D-)2-hydroxyglutarate; IGF, insulin growth factor; MRS, magnetic resonance spectroscopy; MS, mass spectroscopy.

of hypoglycaemia). Prolongation of QTc time is a rare adverse effect of chloroquine and patients will undergo an ECG every 24 weeks. Large cumulative doses (>460 gram) of chloroquine can induce retinopathy (Bull’s Eye maculopathy).322 Daily doses up to 250 mg per day for several years are considered to carry an acceptable risk for chloroquine-induced retinopathies.323 In the present clinical trial, patients will be treated with 200 mg chloroquine per day (cumulative dose per year: 73 grams). Therefore, this clinical trial carries a very low risk to induce chloroquine-related retinopathies. Long-term use of chloroquine (>5 years or >300 grams 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 grams during his/her trial participation.
Chapter 8

**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 \( D2HG \) 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. 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 I/II dose-finding study, this clinical trial does not have a data and safety monitoring board (DSMB).

**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 judgment 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 gastro-intestinal nature.

**Ethics**

This study is being conducted according 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 (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.

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, 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.