Metformin in patients with advanced pancreatic cancer: a double-blind, randomised, placebo-controlled phase 2 trial

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Summary

Background In preclinical work and retrospective population studies, the anti-diabetic drug metformin has been associated with antineoplastic activity and decreased burden of many cancers, including pancreatic cancer. There is therefore interest in the hypothesis that this drug might be repurposed for indications in oncology. We aimed to assess the efficacy of the addition of metformin to a standard systemic therapy in patients with advanced pancreatic cancer, and provide the first report of a clinical trial with a survival endpoint of metformin for an oncological indication.

Methods We did this double-blind, randomised, placebo-controlled phase 2 trial at four centres in the Netherlands. Patients aged 18 years or older with advanced pancreatic cancer were randomly assigned (1:1), via a permutated computer-generated block allocation scheme (block size of six) to receive intravenous gemcitabine (1000 mg/m²) on days 1, 8, and 15 every 4 weeks and oral erlotinib (100mg) once daily in combination with either oral metformin or placebo twice daily. Metformin dose was escalated from 500 mg (in the first week) to 1000 mg twice daily in the second week. Randomisation was stratified by hospital, diabetes status, and tumour stage. The primary endpoint was overall survival at 6 months in the intention-to-treat population. This trial is complete and is registered with ClinicalTrials.gov, number NCT01210911.

Findings Between May 31, 2010, and Jan 3, 2014, we randomly assigned 121 patients to receive gemcitabine and erlotinib with either placebo (n=61) or metformin (n=60). Overall survival at 6 months was 63·9% (95% CI 51·9–75·9) in the placebo group and 56·7% (44·1–69·2) in the metformin group (p=0·41). There was no difference in overall survival between groups (median 7·6 months [95% CI 6·1–9·1] *vs* 6·8 months [95% CI 5·1–8·5] in the metformin group; hazard ratio [HR] 1·056 [95% CI 0·72–1·55]; log-rank p=0·78). The most frequent grade 3–4 toxic effects were neutropenia (15 [25%] patients in placebo group *vs* 15 [25%] in metformin group), skin rash (six [10%] *vs* four [7%]), diarrhoea (three [5%] *vs* six [10%]), and fatigue (two [3%] *vs* six [10%]).

Interpretation Addition of a conventional anti-diabetic dose of metformin does not improve outcome in patients with advanced pancreatic cancer treated with gemcitabine and erlotinib. Future research should include studies of more potent biguanides, and should focus on patients with hyperinsulinaemia and patients with tumours showing markers of sensitivity to energetic stress, such as loss of function of AMP kinase, a key regulator of cellular energy homoeostasis.

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Introduction

There is substantial interest in the hypothesis that the widely used anti-diabetic drug metformin has anti-neoplastic activity.[1] More than 100 clinical trials of this compound for various indications in oncology are now in progress. However, clinical trial results reported so far[2,3] have been from pilot studies with biomarker endpoints.

Many retrospective pharmacoepidemiological studies have suggested that patients with diabetes treated with metformin have a reduced cancer risk, an improved cancer prognosis, or improved survival.[3–5] However, the methods of some of these studies have been criticised, and other reports[6,7] have concluded that no association exists between metformin use and cancer risk or prognosis. Furthermore, retrospective studies have been restricted to patients with diabetes, and the relevance of results to non-diabetic patients with cancer is unknown.

Laboratory studies also provide a rationale for clinical trials of metformin in cancer treatment.[3] There is evidence to suggest that one major action of metformin and other biguanides is partial inhibition of oxidative phosphorylation.[8,9] In models of type 2 diabetes, inhibition of oxidative phosphorylation in the liver, the main target of the anti-diabetic action of metformin, results in hepatic energetic stress, which leads to decreased gluconeogenesis.[10,11] In this way, metformin can reduce serum glucose concentrations and reduce the hyperinsulinaemia associated with insulin resistance.[12] The metformin-induced decrease in insulin represents a potential antineoplastic mechanism because a subset of cancers display insulin receptors and are insulin responsive.[13,14] In addition, although most cancers show increased rates of glycolysis, they also require oxidative phosphorylation.[15] Thus, a separate possible antineoplastic mechanism is that metformin might accumulate in neoplastic tissue at concentrations sufficient to inhibit oxidative phosphorylation and cause energetic stress in transformed cells.[16–18] Laboratory in-vitro and in-vivo models[19,20] have provided evidence not only that pancreatic cancer stem cells are sensitive to inhibition of oxidative phosphorylation, but also that metformin is active in pancreatic cancer. An important caveat is that many in-

Research in context

Evidence before this study

Metformin is a widely used, inexpensive, and non-toxic anti-diabetic drug. Substantial laboratory evidence supports the hypothesis that this compound has antineoplastic activity, and plausible mechanisms of action have been proposed. Pharmacoepidemiologic evidence for antineoplastic activity of metformin is not consistent, but some studies show a reduced risk or improved prognosis of many cancers, including pancreatic cancer, in patients with diabetes treated with metformin. In view of these findings, more than 100 clinical trials of metformin for various indications in oncology have been started, but so far, the only data reported are for surrogate endpoints, such as proliferation rate estimated by Ki67 labelling. We searched PubMed for original research articles and reviews published in English up to Feb 1, 2015, using MeSH terms "pancreatic cancer" and "metformin". We identified no clinical trials. We designed our trial on the basis of available preclinical data and various epidemiological reports.

Added value of this study

To our knowledge, we present the first report of a randomised, placebo-controlled trial of metformin with a survival endpoint for cancer treatment. Our findings show no benefit of the addition of metformin to the combination of gemcitabine and erlotinib in the treatment of patients with advanced pancreatic cancer.

Implications of all the available evidence

Despite laboratory evidence for antineoplastic activity of metformin, conventional anti-diabetic doses of metformin did not improve survival of patients with advanced pancreatic cancer. Many in-vitro studies show direct antiproliferative actions of metformin at millimolar concentrations, but we determined blood metformin concentrations to be in the micromolar range in our study. Thus, future and ongoing studies of metformin and other more bioavailable oxidative phosphorylation inhibitors should include pharmacokinetic and pharmacodynamic endpoints. Our results should not be generalised to other cancers, for which many clinical trials of metformin are underway.

vitro models use metformin concentrations higher than those identified in the plasma of patients treated with conventional anti-diabetic doses of metformin.

Patients with pancreatic cancer have poor 5-year survival. At present, gemcitabine is widely accepted as the standard chemotherapy drug for patients with this disease.[21] Two clinical trials[22,23] changed the standard of care from single-drug gemcitabine to combination therapy, but these regimens are restricted to patients with a good performance status who do not have comorbidities. New drugs such as erlotinib add some benefit to conventional cytotoxic drugs. Despite these advances, new treatment strategies are urgently needed. Although the survival benefit of the combination of gemcitabine and erlotinib in patients with advanced pancreatic cancer is small, this regimen can be regarded as a standard backbone treatment schedule for clinical trials investigating drugs targeting other signalling pathways.[24] Preclinical studies[25,26] have provided evidence that metformin and EGFR tyrosine-kinase inhibitors act synergistically in basal breast cancer and non-small-cell lung cancer.

We did this study to establish whether the addition of metformin to gemcitabine and erlotinib improves the outcome of patients with advanced pancreatic cancer.

Methods

Study design and participants

We did this double-blind, randomised, placebo-controlled, phase 2 trial at four hospitals in the Netherlands (appendix). The study protocol is available online. We recruited patients with measurable, cytologically or histologically confirmed metastatic or unresectable locally advanced pancreatic adenocarcinoma. We did not include patients with borderline resectable disease. Eligible patients were aged 18 years or older, with a WHO performance status of 2 or lower, an estimated survival of at least 2 months, and adequate bone marrow (white blood cell count $>3\cdot0\times10^9$ cells per L, platelets $>100\times10^9$ cells per L), hepatic (bilirubin $<1\cdot5$ times the upper limit of normal [ULN], alanine aminotransferase or aspartate aminotransferase $<5\cdot0$ times the ULN in case of liver metastases and $<2\cdot5$ the ULN in the absence of liver metastases), and renal function (creatinine <150 μmol/L or a creatinine clearance >1 mL/s per 1·73m², or both).

We excluded patients if they were hypersensitive to metformin or had any systemic disorder that would compromise the safe use of the study drugs. Previous gemcitabine-based therapy was not permitted except when given as (neo)adjuvant therapy that was completed at least 6 months before randomisation. Previous treatment with metformin within 6 months before enrolment or with erlotinib was not allowed. If patients had received previous non-gemcitabine-based first-line treatment, they could be included if they had progression according to Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1.

This study was approved by the medical ethical committees of the participating institutions and conformed to the principles of the International Conference on Harmonisation on Good Clinical Practice. All participants provided written informed consent before randomisation.

Randomisation and masking

Hospital pharmacy staff randomly assigned patients in a 1:1 ratio by computer-generated permuted-block

randomisation (block size of six), to receive gemcitabine and erlotinib with either placebo or metformin. Randomisation was stratified by hospital, diabetes (yes *vs* no), and tumour stage (locally advanced *vs* metastatic). The allocation sequence was generated by the TENALEA Clinical Trial Data Management System (Amsterdam, Netherlands) and was held by the hospital pharmacist, who assigned the patients to treatment. Patients, physicians, and study personnel were masked to treatment allocation. Treatment allocation was concealed by keeping block size confidential. The unmasked data became available to the investigators after final database lock (July 22, 2014).

Procedures

Gemcitabine and erlotinib were given according to previously published methods.[24] Gemcitabine (1000 mg/m²) was given by 30-min intravenous infusion on days 1, 8, and 15 of a 4-week cycle. Erlotinib was given orally at a dose of 100 mg daily. Metformin and placebo were administered orally at a dose of 500 mg twice a day and, if well tolerated, increased to 1000 mg twice a day in the second week to conform with the metformin label from the US Food and Drug Administration.[27] Treatment was continued until disease progression, the occurrence of unacceptable or irreversible toxic effects, or withdrawal of patient consent. Doses could be reduced or delayed to allow recovery from toxic effects at the discretion of the local investigator (for more information see protocol). Metformin or placebo could be reduced to 50% of the maximum dose, erlotinib to 50% and 25%, and gemcitabine to 75% and 50%. Doses could be delayed for as long as necessary according to the judgment of the investigator. Patients could discontinue metformin, placebo, or erlotinib if these drugs caused unacceptable or irreversible toxic effects. These patients could continue to use other assigned study drugs. If patients had irreversible or unacceptable toxic effects that prevented the continuation of gemcitabine, all study treatment was discontinued. If patients were diagnosed with diabetes or glucose intolerance and needed treatment during the study, all anti-diabetic drugs, except metformin, were allowed.

We assessed tumour response and progression with RECIST version 1.1 every two cycles using CT scans. We assessed toxic effects weekly in the first two cycles, and at the beginning of every consecutive cycle, using the National Cancer Institute Common Toxicity Criteria version 4.0. We monitored study drug-related toxic effects until they returned to baseline or were deemed irreversible. On July 6, 2011, the medical ethics committee approved an amendment, proposed by SK, RAM and JWW, to allow measurements of metformin trough levels in plasma samples of all patients treated in the AMC, at day 8 of cycle one and day 1 of cycle two. Blood samples were taken 10–16 h after the preceding metformin dose. We determined metformin plasma concentrations at the end of the study with liquid chromatography coupled to mass spectrometric detection (LC-MS/MS) while masked to treatment allocation. The high plasma trough cutoff value of greater than 1·0 mg/L was based on a study[28] in patients with diabetes that suggested this value was at roughly the 75th percentile.

We collected fasting blood samples at baseline and on day 1 of cycle two and determined the concentrations of glucose, insulin, insulin-like growth factor 1 (IGF-1), and IGF binding protein 3 (IGFBP-3) by chemiluminescent immunometric assays with an Immulite 2000 analyser (Siemens Healthcare Diagnostics BV, Netherlands). The intra-assay coefficient of variation was less than 6% and the inter-assay coefficient of variation was less than 9%. We measured insulin, IGF-1 and IGFBP-3 only in those patients treated in the Academic Medical Centre due to logistic reasons. At baseline (within 28 days before first treatment) and during treatment, we measured all other serum analytes (haemoglobin, thrombocytes, leucocytes, CA19.9, HbA_{1c}, electrolytes, glucose) with standard ELISA methods with reagents purchased from Immunodiagnostic Systems (Boldon, UK) or ABCO Diagnostici (L'Aquila, Italy). We measured HbA_{1c} and C-peptide at baseline.

Outcomes

The primary endpoint was overall survival at 6 months, defined as the proportion of patients still alive from the the start of study treatment to the time of death from any cause. Secondary endpoints were progression-free survival (median time from the start of study treatment until disease progression), overall survival (from the start of study treatment until death from any cause), the proportion of patients achieving an objective partial response (defined

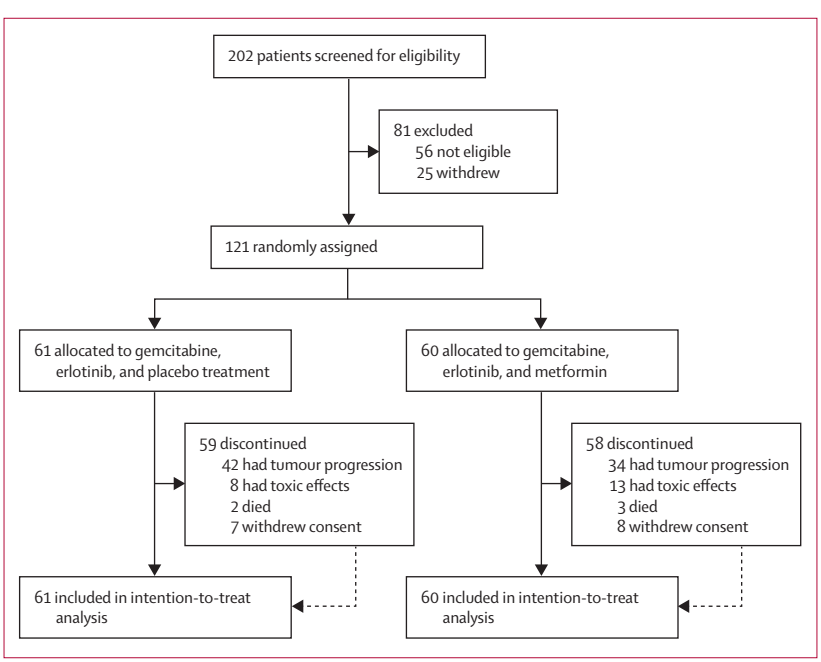

Figure 1: **Trial profile**

as per RECIST v1.1), and safety. An exploratory endpoint was to determine the predictive value of the blood concentrations of metformin, glucose, insulin, IGF-1, and IGFBP-3.

Statistical analysis

It has been suggested that at least a 50% improvement in outcome compared with a standard regimen is needed in a randomised phase 2 trial to support progress to a phase 3 setting.[29] Therefore, to detect an increase in 6 month overall survival from 50%[25] to 75% with the addition of metformin compared with placebo, a sample size of 120 patients was required with a power of 80% and a two-sided α level of 5%.

Overall survival at 6 months and the time-to-event endpoints were estimated with the Kaplan-Meier method, and differences were analysed with the log-rank test stratified by tumour stage and diabetic state. We used χ^2 and Fisher's exact tests to detect differences in overall response and baseline characteristics. We assessed the predictive value of biomarkers by univariate analysis. We assessed the predictive value of changes in biomarker blood concentrations between baseline and before the start of cycle two with repeated measurement ANOVA, which reports the difference between the biomarker concentrations at the two timepoints, and the difference between the placebo and metformin group. We regarded p values of less than 0·05 as significant. We did all outcome analyses in the intention-to-treat population. We did statistical analyses with SPSS (version 21.0). The study was monitored by an independent data safety monitoring committee (IDMC), convened every 3 months. After 20, 60, and 90 patients had been enrolled, the IDMC compared toxicity data between treatment groups. After 90 patients had been enrolled, the IDMC also did an interim analysis for efficacy. This study is registered with ClinicalTrials.gov, number NCT01210911.

	Placebo group (n=61)	Metformin group (n=60)
Age (years, range)	65 (44–79)	64 (45–78)
Sex		
Male	27 (44%)	34 (57%)
Female	34 (56%)	26 (43%)
WHO performance status		
0	33 (54%)	27 (45%)
1	24 (39%)	22 (37%)
2	2 (3%)	8 (13%)
Not documented*	2 (3%)	2 (3%)
Line of treatment of study drug		
First line	59 (97%)	58 (97%)
Second line	2 (3%)	2 (3%)
Disease stage		
Locally advanced	16 (26%)	16 (27%)
Metastatic disease	45 (74%)	44 (73%)
Primary tumour location†		
Head	39 (64%)	40 (67%)
Body	20 (33%)	18 (30%)
Previous surgery		
None	45 (74%)	51 (85%)
PPPD or Whipple	6 (10%)	4 (7%)
Palliative HJS	10 (16%)	5 (8%)
Diabetes	8 (13%)	6 (10%)
Fasting glucose (mmol/L; n=119)	7·3 (2·5)	7·7 (2·6)
HbA_{1c} (mmol/mol; n=105)	47 (12)	45 (10)
Fasting concentrations of serum markers		
Insulin (pmol/L; n=96)	117 (110)	128 (129)
IGF-1 (nmol/L; n=95)	22 (10)	21 (9)
IGFBP-3 (mg/L; n=98)	1·90 (0·43)	1·87 (0·51)
CA19.9 (kU/l; n=119)	245 (21–2118)	561 (112–6319)

Data are median (IQR), n (%), or mean (SD) unless otherwise specified. PPPD=pylorus-preserving pancreaticoduodenectomy. HJS=hepaticojejunostomy. HbA_{1c}=glycated haemoglobin. CA=carbohydrate antigen. *Not documented but lower than 3. †In four patients (two in placebo group and two in metformin group) it was not possible to specify whether the location was head or body.

Table 1: **Baseline characteristics**

Role of the funding source

The funders of this study had no role in the study design, data collection, data analysis, data interpretation, or writing of the report. SK, MJW, DJR, and JWW had full access to all the raw data and the corresponding author (JWW) had the final responsibility for the decision to submit for publication.

Results

Between May 31, 2010, and Jan 3, 2014, we screened 202 patients with advanced pancreatic cancer, and randomly assigned 121 patients to receive gemcitabine and erlotinib with either placebo (n=60) or metformin (n=60; figure 1). At the preplanned interim analyses, the IDMC identified no reason to prematurely end the study for toxic effects or futility. The decisions of the IDMC had no effect on the statistical analyses. Baseline characteristics were balanced between the groups, with the exception of the mean baseline concentration of carbohydrate antigen 19.9, which was roughly two times higher in the metformin treatment group (table 1).

Eight (7%) patients had received previous systemic treatment: two patients received gemcitabine-based (neo)-adjuvant chemoradiotherapy and two patients received adjuvant gemcitabine treatment more than 6 months before study treatment. The study treatment in these patients was regarded as first line. The remaining four patients had received previous systemic treatment for advanced disease, all of which were fluorouracil based (study treatment was considered second line). After study treatment, 28 (23%) patients received second-line treatment: 15 in the placebo group and 13 in the metformin group. Median duration of follow-up was 28·1 months (IQR 20·0–47·6). At database lock, 117 (97%) patients had discontinued the study and four (3%) patients (two in each treatment group) remained on-study (figure 1).

Patients received a median of five (IQR 2·0–6·0) treatment cycles in the placebo group and three (2–6) cycles in the metformin group (p=0·050); the median duration of treatment was 4·6 months (IQR 1·8–7·0) and 2·5 months (1·1–6·0), respectively (p=0·20).

Doses of placebo were reduced less often than those of metformin (17 [28%] of 61 patients *vs* 34 [57%] of 60 patients, respectively, p=0·0020), and placebo doses were escalated more frequently than those of metformin (49 [80%] *vs* 38 [63%] patients; p=0·044; appendix). Drug delivery of gemcitabine and erlotinib were similar between treatment groups (appendix).

The overall survival analysis was based on 106 (88%) deaths in 121 patients; 54 (89%) of 61 patients died in the placebo group and 52 (87%) of 60 patients died in the metformin group (p=0·79). Overall survival at 6 months was 63·9% (95% CI 51·9–75·9) in the placebo group and 56·7% (44·1–69·2) in the metformin group (p=0·41). Median overall survival was 7·6 months (95% CI 6·1–9·1) in the placebo group and 6·8 months (5·1–8·5) in the metformin group (hazard ratio [HR] 1·056 [95% CI 0·72–1·55]; log-rank p=0·78, figure 2). After adjustment for stratification factors, the hazard ratio was 1·06 (95% CI 0·73–1·56). Tumour stage was an independent variable associated with survival. Median progression-free survival was 5·4 months (95% CI 5·0–5·8) in the placebo group and 4·1 months (1·8–6·5) in the metformin group (HR 1·18; 95% CI 0·77–1·82; log-rank p=0·44). Five patients in each group had an objective response (p=1·00). 32 (52%) patients in the placebo group achieved disease control, as did 24 (40%) of those in the metformin group (p=0·20).

Table 2 shows adverse event data. The incidence of grade 1–2 toxic effects differed between groups—eg, vomiting (15 [25%] of 61 placebo patients *vs* 26 [43%] of 60 metformin patients), and grade 1–2 anorexia (12 [20%] *vs* 22 [37%], respectively); however, grade 3–4 adverse events appeared similar between groups (table 3). Haematological toxic effects were common and similar between treatment groups (table 3). We were unable to obtain the number of patients diagnosed with diabetes and glucose intolerance during the study. No patients developed hypoglycaemia.

In the placebo group, eight (13%) of 61 patients discontinued all study drugs because of treatment-related toxic effects. Two (3%) patients had gastrointestinal toxic effects and six (10%) patients had other toxic effects: neutropenia (two patients), thrombotic thrombocytopenic purpura (two), and one case each of infection and fatigue. In the metformin group, 13 (22%) of 60 patients discontinued all study drugs because of treatment-related toxic effects. Six (10%) patients had gastrointestinal toxic effects and seven (12%) patients had other toxic effects: fatigue (three patients) and one case each of thrombotic thrombocytopenic purpura, liver toxicity, pulmonary embolism, and infection.

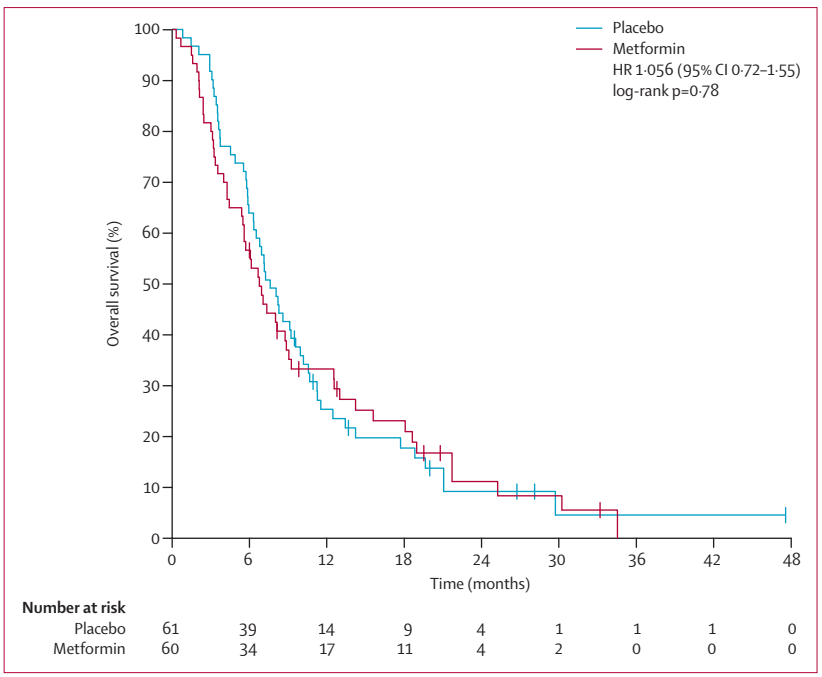

Figure 2: **Kaplan-Meier curves for overall survival**

In the metformin group, toxic effects caused discontinuation of erlotinib alone in two patients (rash), metformin alone in one patient (gastrointestinal toxic effects), and erlotinib and metformin together in four patients (gastrointestinal toxic effects). In the placebo group, no patients discontinued one or two study drugs before the end of the study.

Three patients (two in the placebo group and one in the metformin group) developed gemcitabine-induced thrombotic thrombocytopenic purpura. The course of the disease was moderate-to-severe and self-limiting after discontinuation of treatment.

Following a protocol amendment, we measured the plasma metformin trough concentrations in a subset of patients (n=61) at day 8 of cycle one and day 1 of cycle two. Because of logistical reasons, inadequate blood samples were drawn in two patients, both in the metformin group. These patients were excluded from this part of the analysis. In the patients randomly assigned to metformin (n=29), the mean metformin concentration was 0·48 mg/L (SD 0·33) at day 8 of cycle one and 0·67 mg/L (0·51) at day 1 of cycle two. In 18 (62%) of these 29 patients, dose escalation of metformin to 1000 mg twice a day was possible, which resulted in an increase in the mean metformin concentration. The mean trough concentration at day 1 of cycle two in the escalated-dose group was 0·85 mg/L (SD 0·52) compared with 0·34 mg/L (SD 0·33) in the low-dose group (p=0·015). In a preplanned exploratory analysis, overall survival was significantly longer in the 16 patients with high trough concentrations of metformin (greater than 1·0 mg/L) on day 1 of cycle two than in the 13 patients with low trough concentrations

	Placebo group (n=61)			Metformin group (n=60)		
	Grade 1–2*	Grade 3	Grade 4	Grade 1–2*	Grade 3	Grade 4
Haemoglobin decreased	52 (85%)	2 (3%)	0	42 (70%)	2 (3%)	0
Neutropenia	19 (31%)	12 (20%)	3 (5%)	16 (27%)	14 (23%)	1 (2%)
Platelet count decreased	36 (59%)	3 (5%)	0	37 (63%)	4 (7%)	0
Nausea	31 (51%)	1 (2%)	0	34 (57%)	3 (5%)	0
Diarrhoea	27 (44%)	3 (5%)	0	31 (52%)	6 (10%)	0
Fatigue	29 (48%)	2 (3%)	0	31 (52%)	6 (10%)	0
Skin rash	38 (62%)	6 (10%)	0	28 (47%)	4 (7%)	0
Vomiting	15 (25%)	2 (3%)	0	26 (43%)	2 (3%)	0
Anorexia	12 (20%)	1 (2%)	0	22 (37%)	0	0
Mucositis	11 (18%)	0	0	9 (15%)	1 (2%)	0
Constipation	11 (18%)	0	0	7 (12%)	0	0
Weight loss	5 (8%)	0	0	7 (12%)	0	0
Alopecia	14 (23%)	2 (3%)	0	6 (10%)	0	0
Oedema	7 (11%)	1 (2%)	0	5 (8%)	0	0

*Reported in 10% or more of patients.

Table 2: **Treatment-related adverse events according to treatment group**

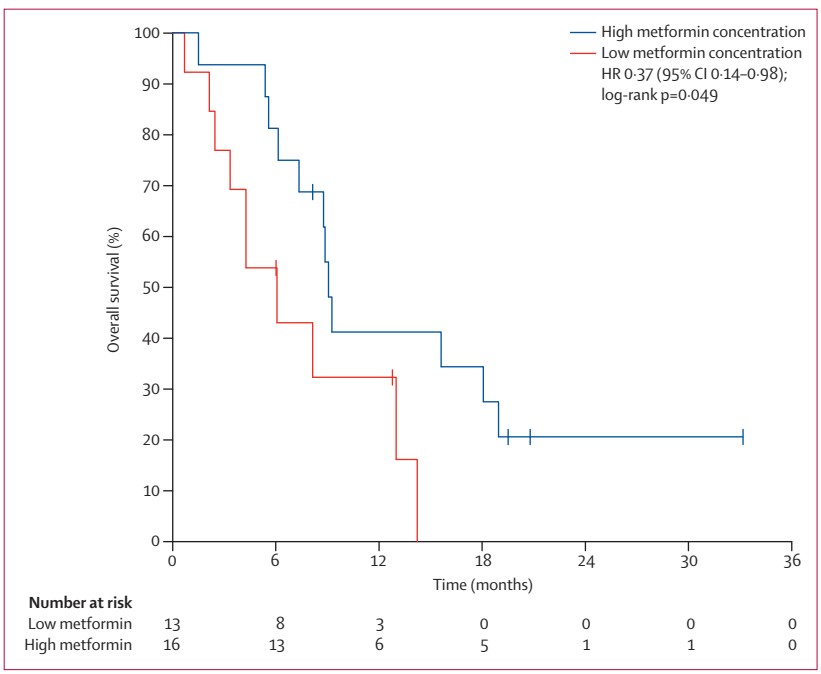

Figure 3: **Overall survival according to metformin concentration**
A high metformin concentration was at least 1 mg/L.

(median overall survival 9·1 months [95% CI 8·3–9·8] *vs* 6·1 months [3·1–9·1]; figure 3). There was no significant difference in overall survival between patients in this subset receiving placebo (median overall survival 7·3 months [95% CI 5·0–9·5]) and patients with low metformin concentrations (6·1 months [3·1–9·1]; HR 0·74, [95% CI 0·33–1·65], p=0·40) or patients with high metformin concentrations (9·1 months [8·3–9·8]; 1·37 [0·71–2·66], p=0·26).

We measured the concentration of glucose in all patients and the concentrations of insulin, HbA_{1c}, IGFBP-3, and IGF-1 in all patients treated in the Academic Medical Centre (n=107), at baseline, day 8 of cycle one and day 1 of cycle two. Because of logistical reasons, inadequate blood samples were drawn for some or all of the analytes in 12 patients (nine in placebo group and three in metformin group). Patients with no available data for any of the analytes were excluded from this part of the study.

Mean baseline concentrations of glucose, insulin, HbA_{1c}, IGFBP-3, and IGF-1 were similar between the placebo and metformin group (table 1). Baseline concentrations were neither prognostic nor predictive for overall survival (data not shown). We did a post-hoc analysis to correlate C-peptide concentrations to insulin concentrations and body-mass index. Mean C-peptide concentrations were highly correlated with insulin concentrations in all patients (Spearman correlation coefficient (ρ)=0·762; p<0·0001). Higher insulin (p<0·0001) and C-peptide concentrations (p=0·0060) were associated with an increased body-mass index.

Table 3 shows the results of a post-hoc repeated measurement ANOVA comparing the change in the mean biomarker concentrations between baseline and day 1 of cycle two. Only patients with available data at both timepoints were included in these analyses. Differences in the change in glucose and insulin concentrations were not significant between the treatment groups (table 3).

In an exploratory analysis, those patients in the metformin group who had a decrease in insulin concentrations between baseline and the first day of cycle two (n=12) had a better overall survival (18·6 months, 95% CI 8·5–28·7) versus those who did not have increased insulin concentrations (n=10; 5·7 months [95% CI 3·9–7·5]; HR 0·20 [95% CI 0·06–0·60], p=0·004). In the placebo group, the patients who had a decrease in insulin concentrations (n=18) showed no difference in survival (7·2 months [95% CI 4·3–10·2]) versus those who did not have increased insulin concentrations (n=11, 7·6 months [95% CI 5·1–10·1]; HR 1·12 [95% CI 0·51–2·50]; p=0·776; figure 4). However, these exploratory results must be interpreted with caution given the small number of patients in each group.

Discussion

This phase 2 trial suggests that the addition of a conventional anti-diabetic dose of metformin to gemcitabine and erlotinib does not improve the clinical outcome of unselected patients with advanced pancreatic cancer.[30] To our knowledge, this is the first randomised, double-blind, placebo-controlled trial of metformin in cancer treatment with a survival endpoint. Strengths of our study compared to other clinical trials of metformin reported so far include the randomised placebo-controlled design and the use of clinical endpoints rather than biomarker surrogates. However, we recognise that our trial was not comprehensive in the assessment of serum

	Placebo group				Metformin group				Between groups
	Patients (n)	Baseline	Cycle two	p value	Patients (n)	Baseline	Cycle two	p value	p value
Glucose (mmol/L)	52	7·3 (2·5)	7·3 (2·4)	0·96	47	7·7 (2·8)	7·1 (2·4)	0·060	0·72
Insulin (pmol/L)	29	103 (102)	110 (88)	0·81	22	84 (95)	69 (51)	0·38	0·34
IGF-1 (nmol/L)	27	22 (12)	17 (8)	<0·0001	23	23 (10)	19 (9)	<0·0001	0·92
IGFBP-3 (mg/L)	28	1·91 (0·47)	1·76 (0·47)	<0·0001	24	1·91 (0·51)	1·69 (0·56)	<0·0001	0·80

Data are mean (SD) unless otherwise stated.

Table 3: **Concentrations of biomarkers and statistical difference between baseline and cycle two and between treatment groups**

biomarkers, did not include characterisation of tumour biomarkers, and was not designed to detect a small survival benefit or a benefit confined to a small subset of patients with pancreatic cancer. An attempt at voluntary retrieval of consecutive tissue samples during treatment did not succeed because of difficulties in the procedure, low tumour yields, and low patient participation. Patient heterogeneity was introduced because of the inclusion of patients with locally advanced and metastatic tumours, which is not uncommon in phase 2 trials of patients with pancreatic cancer. The results were probably not affected because the endpoints were adjusted for the stratification factors.

We chose a phase 2 design because our main intent was to study the efficacy of metformin when added to standard treatment. When we started this trial, little high-level evidence for metformin's efficacy was available. Thus, we needed a reasonable estimate of metformin's efficacy before a phase 3 trial could be justified. Many phase 3 trials testing new gemcitabine-based regimens in patients with advanced pancreatic cancer have not shown improvements in survival, despite promising results at phase 2. Thus, large differences in outcome should be identified in phase 2 studies before proceeding to phase 3 trials.[29] We therefore aimed to detect an increase in overall survival at 6 months from 50% to 75%.

We chose a 6 month survival endpoint even though a time-to-event approach is commonly used in oncological trials because we postulated that metformin treatment would not lead to a cure but only to a delay in mortality. Time-to-event approaches are often based on the assumption that a constant effect is present and we postulated that the maximum difference between the cumulative hazards would be around 6 months, which is roughly the median survival of the patient population. Indeed, a cross-trial comparison, which should be interpreted with caution, showed that the median overall survival of our control group (7·6 months [95% CI 6·1–9·1]) had a 95% CI that overlapped with the 95% CI of the median overall survival from the control groups of other randomised trials.[22–24] Although the standard comparison group in those trials was gemcitabine monotherapy, we added erlotinib because preclinical evidence showed synergism between EGFR inhibition and

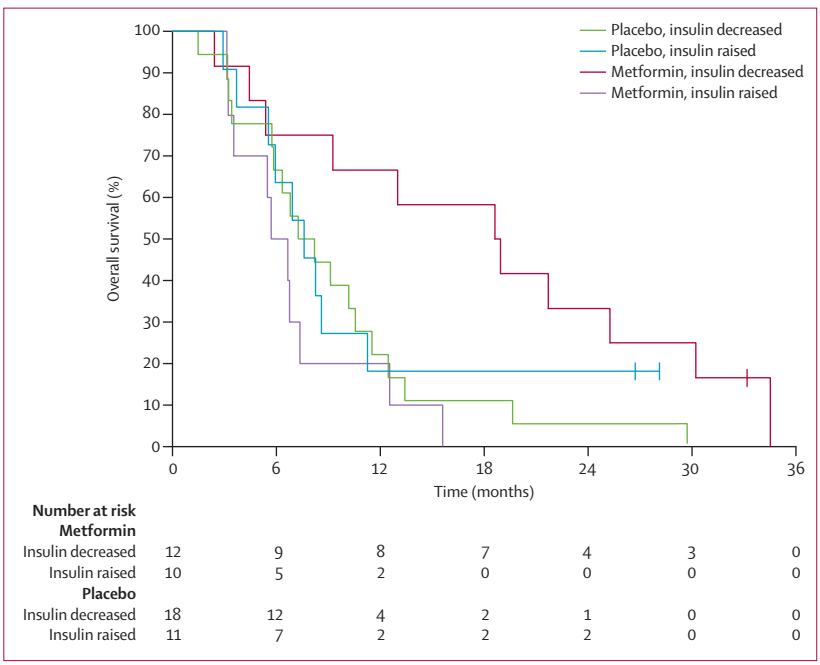

Figure 4: **Overall survival according to insulin concentration change**

metformin.[25,26] Such an effect is unknown for the newer treatment regimens such as FOLFIRINOX and gemcitabine plus nab-paclitaxel.

Retrospective population studies have suggested that metformin might have benefits in cancer prevention or treatment. Our trial supports the conclusions of pharmaco-epidemiological research that questions the methods of some of the earlier reports and contradicts their conclusions.[6,7]

How can the negative results of our clinical trial be interpreted in the context of the previous encouraging laboratory evidence? With respect to the proposed direct action of metformin on cancer cells, the drug concentrations achieved in neoplastic tissue are crucial, and conventional anti-diabetic doses of metformin might fail to accumulate to a sufficient concentration to cause energetic stress. Many in-vitro models showing anti-neoplastic activity of metformin use drug concentrations in the millimolar range. By contrast, plasma metformin

concentrations in our study were in the 0·5–1 mg/L (about 7 μmol/L) range, similar to plasma concentrations seen in patients with diabetes treated with the same dose of metformin.[28] Patients with high plasma concentrations (>1 mg/L) of metformin seemed to have an improved survival. Although this finding might suggest an association between plasma drug concentration and outcome it comes with the caveat that metformin dose escalation was less likely in patients with more advanced disease and gastrointestinal symptoms who had a worse prognosis. Nevertheless, because we measured trough metformin concentrations in blood samples that were not precisely timed in terms of hours since preceding dose, these data might underestimate any association between the dose received and peak or trough drug concentrations, and between achieved plasma concentration and probability of survival. Whether higher dosages could have been reached, in light of the higher frequency of gastrointestinal toxic effects and dose reductions in the metformin group compared with the placebo group, is questionable. We decided not to include a run-in period to assess the optimum dose of metformin because of the extensive experience with this drug in patients with diabetes in whom doses of more than 2550 mg per day results in unacceptable toxic effects, and the epidemiological data suggesting that the anti-diabetic dose has an effect on pancreatic cancer.[4,5,27]

Another factor that might restrict the direct action of metformin on neoplastic tissue compared with liver tissue (a key target tissue for the anti-diabetic action of the drug), is the expression of the cell-surface transport proteins needed for metformin entry[31]—hepatic expression of these proteins is high but expression in tumours is variable. Furthermore, the liver is exposed via the portal circulation after oral dosing to drug concentrations that are higher than concentrations in the systemic circulation. If drug entry into neoplastic cells is indeed a limiting factor, the use of biguanides other than metformin, such as phenformin, which are more lipophilic and less dependent on active transport, might have a therapeutic advantage. Although phenformin is more toxic than metformin and offers no advantage for treatment of diabetes, it is less dangerous than many anticancer drugs and represents one example of a compound targeting oxidative phosphorylation that might have advantages compared with metformin for cancer treatment.[32,33]

The proposed indirect mechanism of action of metformin as an antineoplastic drug postulates that its effects are dependent on changes in the host environment, such as a decrease in insulin concentration and a resulting reduction in the activity of the insulin receptor-PI3K-mTOR signalling pathway in neoplastic tissue.[1,3] However, not all cancers are responsive to insulin, and for the subset that are, achieved reductions in insulin concentration might not be sufficient for an antineoplastic effect. A phase 3 study that assessed the effect of a somatostatin analogue on insulin and IGF-1 concentrations as a treatment for breast cancer shows that the intervention caused a significant but small decline in insulin concentrations that was not associated with any clinical benefit, leaving unanswered the question of whether substantial declines in insulin would have an effect.[34] Furthermore, a phase 3 study of an extracellular inhibitor of the IGF-1 receptor, ganitumab, likewise showed no improvement in survival in patients with metastatic pancreatic cancer.[35] Although we did note improved survival in the subset of patients in the metformin group who achieved decreases in insulin, insulin reductions of the same order of magnitude in the placebo group were not associated with a survival benefit. This finding warrants further study; it might be a chance result related to the small number of patients in the subsets, but we cannot exclude the possibility that a difference exists between metformin-induced decreases in insulin and declines associated with disease progression that restricts caloric intake. If the indirect mechanism of action of metformin in laboratory models is clinically relevant, it would be expected to operate preferentially in situations of hyperinsulinaemia, such as in obese patients or in men receiving androgen deprivation therapy for prostate cancer.

In conclusion, our trial shows no advantage for the addition of metformin to erlotinib and gemcitabine in the treatment of advanced pancreatic cancer. Although this result should not be extrapolated to other potential indications for biguanides in oncology, it does draw attention to the fact that ongoing trials are assessing conventional anti-diabetic doses of metformin, which have not been shown clinically to inhibit oxidative phosphorylation in neoplastic tissue. Preclinical research, carried out after this trial was designed, has provided substantial further support for the hypothesis that inhibition of oxidative phosphorylation might be a useful metabolic treatment for cancer, especially for tumours that are hypersensitive to energetic stress due to loss of function of AMP kinase or other control systems that regulate cellular energy metabolism.[15–18,20,29,36,37] Future research in this area should explore more potent inhibitors of oxidative phosphorylation than metformin and include pharmacodynamic assessment of the effects of treatment on mitochondrial function.

Contributors

DJR and JWW contributed to the conception and design of the study. SK, MNP, MJW, AB, CJP, DJR, and JWW were responsible for data acquisition. SK, AHZ, and RAM analysed the data. SK, MNP, AHZ, DJR, and JWW intepreted the data. All authors reviewed and provided input to the outline and manuscript drafts, and provided final approval for manuscript submission.

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