FINAL MANUSCRIPT

Metformin as an adjunctive therapy for pancreatic cancer: a review of the literature on its potential therapeutic use

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Abstract

Pancreatic ductal adenocarcinoma has the worst prognosis of any cancer. New adjuvant chemotherapies are urgently required, which are well tolerated by patients with unresectable cancers. This paper reviews the existing proof of concept data namely: laboratory, pharmacoepidemiological, experimental medicine and clinical trial evidence for investigating metformin in patients with pancreatic ductal adenocarcinoma. Laboratory evidence shows metformin inhibits mitochondrial ATP synthesis which directly and indirectly inhibits carcinogenesis. Drug-drug interactions of metformin with proton pump inhibitors and histamine H2-receptor antagonists may be of clinical relevance and pertinent to future research of metformin in pancreatic ductal adenocarcinoma. To date, most cohort studies have demonstrated a positive association with metformin on survival in pancreatic ductal adenocarcinoma, although there are many methodological limitations with such study designs. From experimental medicine studies, there is a sparse data in humans. The current trials of metformin have methodological limitations. Two small randomized controlled trials (RCTs) reported null findings, but there were potential inequalities in cancer staging between groups and poor compliance with the intervention. Proof of concept data, predominantly from laboratory work supports assessing metformin as an adjunct for pancreatic ductal adenocarcinoma in RCTs. Ideally more experimental medicine studies are needed for proof of concept. However, many feasibility criteria need to be answered before such trials can progress.

Keywords:

Pancreatic cancer, metformin, survival, proof of concept

Introduction

Pancreatic ductal adenocarcinoma (PDAC) has the worst prognosis of any cancer with an overall 5-year survival of less than 5%, which has remained relatively unchanged for several decades.[1] Worldwide, there are 337,872 new cases diagnosed annually, with the incidence similar to the mortality.[1] Surgical resection remains the sole potential curative treatment, although this is only possible in up to 10% of patients who have localized tumors.[2] Those with unresectable PDAC are referred for palliative and supportive care with, interventions focused on symptom control. Palliative chemotherapy with gemcitabine helps alleviate symptoms but gives only a minimal survival benefit, of only 1 month.[2] A more recent chemotherapy regime, FOLFIRINOX, which consists of: oxaliplatin, irinotecan, fluoruracil and leucovorin, increases the median overall survival time to 11.1 months, compared to 6.8 months in patients prescribed gemcitabine ($p<0.001$).[3] For both chemotherapies, particularly FOLFIRINOX, a good medical performance status is required. New chemotherapies are now urgently required, which are well tolerated by many patient groups, to increase survival times for those with this aggressive cancer. A possibility is the oral hypoglycemic drug metformin, due to its emerging additional anti-cancer properties as demonstrated in laboratory experiments.

The purpose of this paper is to review the evidence required to justify the conduct of well-designed RCTs assessing metformin as a treatment in patients with inoperable PDAC. Although several clinical trials have already investigated metformin and reported no benefits, we argue these had methodological and clinical limitations which may have masked any potential benefits of the drug. We suggest further trials, addressing these deficiencies, are now required to more fully assess metformin as a potential chemotherapeutic agent. The feasibility questions which must first be

answered before commencing such trials are also discussed. The article then progresses to review the potential anti-cancer molecular mechanisms of metformin and the pharmacoepidemiological data assessing associations with its use and survival which justify the conduct of further trials.

Methodology

For this narrative review, we searched PubMed using the MESH terms “Metformin” and “Pancreatic Neoplasms”, pharmacoepidemiological studies and clinical trials investigating metformin use and survival in PDAC. The references of papers were reviewed to identify additional papers not found in the initial search. Clinical trials currently being undertaken were identified from ClinicalTrials.gov. A further search identified randomized controlled trials investigating metformin in cancers at other anatomical sites.

Randomized controlled trials

To date, to the best of our knowledge, there have only been two reported RCTs which assessed metformin as a potential adjunctive treatment in patients with inoperable PDAC. In the largest, from the Netherlands, 202 patients were screened for eligibility in 4 hospitals of which; 81 (40%) were ineligible or withdrew their consent**.** Therefore, 121 patients were randomized to receive either: gemcitabine/erlotinib plus oral metformin (n=60) (metformin 500mg twice daily in the first week, escalated to 1000mg twice daily thereafter) or gemcitabine/erlotinib and placebo (n=61).[4] The exclusion criteria included: previous metformin use within 6 months before enrolment, borderline resectable cancer, WHO performance status ≥3 and liver enzymes >5.0 times the upper limit of normal due to liver metastases. In the intention to treat analysis, there was no statistically significant difference in *overall survival* at six months between the

two groups (median 7·6 vs 6.8 months, log-rank test p=0.78, HR 1.06, 95% CI 0.72-1.55). However, there were several reasons which may affect the validity of this null finding. Firstly, there were large differences in the baseline tumor marker CA19-9 levels between the metformin and placebo groups. In the active arm, the median CA19-9 (kU/l) was 561 (IQR = 112-6319) but the level was lower in the control arm at 245 (IQR 21-2118, no p-value quoted). CA19-9, although it has poor sensitivity, may reflect tendency to progression. CA19-9 can be falsely negative in patient groups with a negative Lewis blood group (Le α-β-) antigen phenotype, which is approximately 5-10% of the population.[5,6] However it is possible that more patients allocated metformin had more cancers with a tendency to progress than those who received placebo. This imbalance may mean any potential benefit of the drug went undetected if the metformin group had proportionately more advanced cancers.

Secondly, in the metformin arm, 22% of patients (n=13) discontinued the drug because of a higher proportion of side effects namely: vomiting (43% vs 25%), severe diarrhea (10% vs 5%) and anorexia (37% vs 20%, no p-values quoted). Therefore, more patients stopped metformin than placebo (22% vs 13% p=0.21). In the intention-to-treat analysis, again this may mask any potential therapeutic benefit of metformin if the drug was not actually tolerated and absorbed. The authors did not report a per protocol analysis, namely one in patients who tolerated the drug. Whether these symptoms are side effects of metformin itself or representative of the underlying cancer itself are uncertain, particularly as there were more aggressive cancers in the active drug arm. Symptoms, such as vomiting, could be due to PDAC itself, for example if the cancer invades the duodenal wall giving upper gastrointestinal tract obstruction. If so, the drug is less likely to reach the small intestine where it is absorbed. Furthermore, steatorrhea may occur due to pancreatic enzyme insufficiency

initiated by exocrine destruction of the gland by the cancer. Lastly, patients in the placebo arm received a median of five (IQR 2.0–6.0) cycles of chemotherapy, but only three (IQR 2.0–6.0) cycles in the metformin group (p=0·05). Doses of placebo were reduced less often than metformin (28% *vs* 57% of patients, respectively, p=0·002). Additionally, doses of placebo were escalated more frequently than metformin (80% *vs* 63% of patients, p=0·044). These dose alterations would also bias the results towards the null. The calculated effect size to be detected was perhaps unrealistically high, namely a 6-month overall survival absolute benefit of 25% with 120 patients.

A second and open-label, single center, prospective, smaller randomized phase II trial of 60 patients conducted in Italy, investigated metformin in combination with a chemotherapy regime of: cisplatin, epirubicin, capecitabine and gemcitabine (PEXG), versus PEXG alone, in patients with metastatic PDAC.[7] Here 70 patients were screened for eligibility and 86% (n=60) were randomized. In total, 31 patients were allocated to the PEXG and metformin (2g oral daily) arm, with 29 to the control one prescribed PEXG and placebo. Again, several baseline variables were incomparable between the two groups, namely CA19-9 levels and cancer stage. Here, the median CA19-9 (IU/mL), although not statistically significantly different, was almost half in the metformin arm (457, IQR 33-1962) than in the control one (863, IQR 113-1473) (p=0.66). More patients in the control arm had lung metastasis than in the active one (24% vs 6.5%, p=0.08). The primary end point was *cancer progression-free survival* (PFS) at six months, defined as no evidence of radiological progression or death. In the intention-to-treat analysis, median PFS for the metformin and control arms were 4.9 months vs 6.1 months respectively (HR 2.0, 95% CI 1.05–3.8; *p*=0.036) with no difference in the median *overall survival* between the metformin and control arms (10.4 months vs 6.8 months (HR 0.92, 95% CI 0.54-1.56)). The trial was powered assuming

a baseline PFS of 50% at 6 months, and >70% for the metformin one. To detect this large effect size 78 patients were required (39 per treatment arm) so the trial only recruited 77%, of those required to detect this large effect size. The significance level used was 10% i.e. a 10% chance of detecting a false positive result, which is above conventional full statistical significance. This relatively small sample size and the imbalances in characteristics between groups mean definitive conclusions on metformin's efficacy cannot be reached. The authors reported there were no differences in grade 3 or 4 toxic effects in the patients assigned metformin. Grade 3 adverse events are classed as *severe*, but not immediately life-threatening events requiring hospitalization, and grade 4 adverse events have *life threatening* consequences. The authors did not report the frequency of less serious grade 1 or 2 toxic events, although they state only two patients withdrew IMP due to drug-related side effects. Additionally, the authors made no comment on why the trial was open-label, and not double-blinded.

For both these trials we assessed bias using the Cochrane risk of bias tool (Table 1). This consists of seven domains of methodology to minimize bias namely: random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective reporting and other bias. For each domain, the assessor assigns a judgement of *high* or *low risk* of bias. If there is not sufficient evidence available, an *unclear risk* of bias is allocated. Both trials had some inherent biases present when assessed. The Netherlands trial had 2 domains identified as *high risk* of bias, namely incomplete outcome data and other bias. The Italian study had 3 domains which were *high risk* namely, blinding of participants and personnel, blinding of outcome assessment and other bias.

To conduct a large RCT which addresses the limitations inherent in the previous trials, feasibility work must be performed to answer unknowns to both justify and inform the planning of such a large interventional study. Feasibility work must record the proportion of the study population already prescribed metformin at diagnosis, who would be ineligible for randomization, the prevalence of drug side effects and other drugs which may affect its pharmacokinetics. These will help guide planning for the sample size calculations and recruitment into future, large RCTs; considering the drop-out rate during the trial period.

In summary, the null findings from both these RCTs must be interpreted with caution and future trials must address their limitations. As the randomization led to inequalities of cancer staging distribution in opposite directions and both showed no effect of metformin, these could reflect the true result. However, there is another possibility that these inequalities lead to incorrect findings. Larger sample sizes are needed so there are no inequalities in the cancer stages between the two arms, and measures incorporated to ensure drugs are not discontinued. These trials could progress informed by the *proof of concept* data discussed later for assessing metformin as an adjunctive therapy in PDAC.

The pharmacokinetics and pharmacodynamics of metformin

An appreciation of the absorption, metabolism, excretion and actions of metformin is important for developing clinical trials of its use. The biguanide, metformin (N, N-dimethylbiguanide), is a drug commonly prescribed for the management of patients with type 2 diabetes mellitus (T2DM). Discovered in 1922, it was introduced in clinical medicine to treat diabetes in France in 1957[8] and is now on the list of the World Health Organization (WHO) essential medicines.[9] Although metformin is generally

well tolerated, common side effects include gastrointestinal symptoms, namely nausea, vomiting and diarrhea. Rarely, but more seriously, lactic acidosis can occur, which is more likely with existing underlying renal disease.[10]

The absorption of metformin in the small intestine is primarily mediated by the plasma membrane monoamine transporter (PMAT) and organic cation transporter 3 (OCT3).[11,12] OCTs are molecules in the membrane themselves that facilitate movements of endogenous substrates such as creatinine and dopamine into cells.[13] Metformin is not metabolized in the body and has a half-life of approximately 5 hours.[14]. The uptake into hepatocytes is mediated by OCT1 and to a lesser extent by OCT3[14], whereas renal uptake is by OCT 2.[15] Metformin is excreted unchanged through the kidney.[14] Drugs that inhibit these membrane transporters may therefore be clinically relevant. In vitro studies suggested that PPIs inhibit OCT1, OCT2 and OCT3, subsequently raising plasma levels of metformin.[16] This, at first, appears paradoxical as metformin absorption in the intestine would be inhibited. However, it is more likely the effect of PPIs in reducing hepatic absorption of metformin via OCT1 overrides that of reduced intestinal absorption.[12,16] As metformin's passage into hepatocytes is reduced, this may be clinically relevant as the potential anti-cancer action of metformin may occur through inhibition of hepatic gluconeogenesis. Furthermore, the histamine H2-receptor antagonist, cimetidine, through reducing the renal tubular secretion of metformin, increases plasma levels of metformin.[17]

Genetic factors may influence the efficacy of metformin. Approximately 35% of patients prescribed metformin monotherapy for T2DM will fail to achieve optimal glycemic control and require additional hypoglycemic drugs.[18-21] This heterogeneity is partly due to genetic factors resulting in variation of plasma metformin levels, a phenomenon known as pharmacogenomics. Genome-wide association research

which investigated the variability in clinical response to metformin identified the potential responsible genes,[22,23] including the SNP rs11212617 at a locus containing the ataxia telangiectasia mutated gene (ATM).[24,25] The minor allele C of ATM is required for metformin action in the liver by upregulating AMPK activation.[22] More recently, studies have shown ATM also mediates uptake of metformin by hepatic transporters, in particular OCT1.[26,27] Those with minor allele C may therefore respond better to metformin, which is present in 44% of people.[28] Investigating such genes is required to potentially augment metformin's therapeutic actions and mitigate its adverse ones. Such work is relevant to PDAC, to ascertain which patients may best respond to metformin.

Proof of concept

Support for evaluating metformin in appropriately designed RCTs is informed by *proof of concept* data. This may be derived from: i) laboratory studies, ii) epidemiological data, iii) experimental medicine studies in patients, and iv) large RCTs of metformin in other cancers.

Proof of concept 1: Biological plausibility of metformin as an adjunctive chemotherapeutic drug demonstrated in laboratory studies

Metformin may be beneficial in patients with PDAC firstly due to its *direct* inhibitory effects on cancer cells themselves, and secondly its *indirect* actions by lowering blood glucose through inhibiting hepatic gluconeogenesis and increasing glucose utilization in skeletal muscle. A lower blood glucose leads to less potentially mitogenic excess insulin being secreted from pancreatic islet cells and then stimulating cell division.[29] Firstly, metformin may exert direct anti-cancer effects on cells themselves by inhibiting mitochondrial ATP synthesis. This promotes cancer cell death as there is then

insufficient energy for anabolic processes, such as structural protein formation.[30,31] Metformin inhibits complex 1 in the mitochondria, so ATP production decreases, which then increases both cellular AMP:ATP and ADP:ATP ratios which activates the enzyme AMPK.[32] The potential anti-cancer properties of stimulating AMPK are the inhibition of macromolecule synthesis necessary for cell growth and division.[33,34] AMPK also inhibits the mechanistic target-of-rapamycin complex (mTORc) which is responsible for activating numerous cellular pathways, including protein translation.[35,36] AMPK inhibits mTOR through phosphorylation of the Tuberous Sclerosis Complex 2 (TSC2).[35] These cellular processes are summarized in figure 1. Secondly the possible indirect anti-cancer effects of metformin are through reducing secretion of insulin. Excess insulin is mitogenic which may be particularly relevant to pancreatic carcinogenesis, as the hormone is synthesized in, and released from the pancreas itself.[37] Mitogenesis is stimulated by upregulation and activation of the Ras-Raf-mitogen-activated protein (MAP) kinase signaling pathway.[38] Metformin through activating AMPK reduces plasma glucose and consequently mitogenic insulin levels.

In vitro work shows metformin exerts anti-tumor properties on pancreatic cancer cell lines, including inhibiting cell proliferation and apoptosis in a dose-dependent manner.[39] Metformin down-regulates transcription factors (pancreatic duodenal homeobox-1 (PDX-1)) which are related to PDAC.[40] More recently, metformin, when combined with gemcitabine, enhanced the induction of pancreatic cancer cell apoptosis and inhibited cellular proliferation both in vitro and in vivo.[41] A limitation of the current chemotherapy drugs is that drug resistant tumor cells develop, likely mediated by tumor initiation cells (cancer stem cells).[42,43] Metformin inhibited the

function of such $CD44^{+}/CD24^{10}$ cells and these actions were enhanced when the drug was an adjunct to doxorubicin.[44,45]

Laboratory studies assessing the potential anti-cancer effects of metformin have been extensive, but not exclusive to PDAC. In vitro studies have investigated numerous cancers, including: breast,[46-49] prostate,[50] endometrial[51] and brain.[52] The dosages of metformin used were much higher than those prescribed in clinical practice. Nevertheless, there is some laboratory evidence in breast cancer of anti-cancer properties within the normal therapeutic dosage (6μM–30μM) for patients taking metformin for T2DM.[47] In breast cancer cells, a further pathway of action of the possible anti-cancer effects of metformin has been recognized. Breast cancer cells overexpressing human epidermal growth factor receptor 2 (HER2) were inhibited by metformin through direct inhibition of p70S6K1 enzyme activity, independent of the AMPK pathway.[53] p70S6K1 activity is responsible for protein synthesis and cell proliferation and is a downstream target for mTOR.[54] In vivo studies in transgenic mice demonstrated metformin delayed the growth of breast cancer, reduced tumor size [55] and delayed tumor onset,[56] with similar findings in lung cancer and colorectal cancer.[57,58] In conclusion, these laboratory-based studies provide supportive proof of concept information that metformin merits investigation in further trials in PDAC.

Proof of concept 2: Pharmacoepidemiological evidence

Further RCTs of metformin would be supported by pharmacoepidemiological studies showing its use is associated with increased survival times in patients with PDAC after diagnosis, compared to those not prescribed metformin. The observational study design chosen is crucial when appraising the validity of the findings of such work. A

prospective study design would be the most robust, however a further three themes must be considered when assessing the validity of pharmacoepidemiological studies namely: consideration of time-related biases, the choice of reference groups and completeness for data collection on drug use.

i. In pharmacoepidemiological studies assessing post-diagnosis metformin use mitigating against several time-related biases is vital, as if not considered these may lead to spurious over-estimates of the potential benefits of metformin. Immortal time bias is the misclassification of exposed person-time for metformin users. This occurs when exposure time is incorrectly recorded from the date of diagnosis rather than that of the first drug prescription, which may be much later after diagnosis. This bias leads to an overestimate of the length of metformin prescription. The second bias, time window bias, is commoner in retrospective work where participants, taking and not taking metformin have not been matched according to similar durations of the exposure time for potentially receiving the drug. Patients prescribed metformin may have lived longer due to the less aggressive tumors and therefore be more likely to have had the opportunity to be prescribed metformin. The association with the increased survival and metformin is therefore due to the more favorable prognosis of the cancer rather than metformin itself. The ideal pharmacoepidemiological study would consider both time-related biases in their methodology and analysis.

ii. A difficulty with interpreting pharmacoepidemiological studies investigating oral hypoglycemic drugs in PDAC is related to the choice of the reference group i.e. the non-metformin users. One could infer either a potential beneficial effect for metformin, if there is no effect on cancer survival with the reference group i.e. firstly T2DM patients prescribed an alternative oral hypoglycemic drug such as a

sulfonylurea, or secondly patients with PDAC but not T2DM. Using both these groups have potential methodological problems. Hypothetically, drugs such as sulfonylurea could worsen survival as they stimulate insulin release from the pancreas, which in excess is mitogenic.[59] Therefore, an alternative explanation to a beneficial effect of metformin is actually a detrimental one of sulfonylureas, if there is actually no effect of metformin. Using a reference group without T2DM may be problematic if diabetes itself affects survival.

iii. A further methodological problem is due to the short survival times of most patients with inoperable PDAC and how post-diagnosis medication is recorded from the drug databases. When patients are admitted to hospital, the primary care prescription records are incomplete, as the admitting hospital dispense the medications during admission. These interruptions inherent in the primary care records compromise the validity of the data, particularly if the patient has multiple hospital admissions.

Therefore, limitations of the methodology of such pharmacoepidemiological work are potentially large, hence they are difficult to interpret if all these areas are not considered.

In total, 11 retrospective cohort and 1 prospective cohort study were identified (table two). The prospective cohort investigation of 44 patients reported null findings for metformin use (HR 0.70, 95% CI 031-1.59 p=0.39). In the 11 retrospective cohort studies (cohort size range 237-3393), 7 reported statistically significant associations for metformin use improving survival in PDAC. The other 4 reported null findings, although 3 documented statistically significant results in subgroup analyses.

Four studies did clearly define metformin exposure and their actions to mitigate against time-related biases,[60-63] however most were unclear when addressing such bias. Only two studies performed analyses investigating *post-diagnostic* metformin use, although this was not the primary outcome for any of these.[61,62] Of these, one investigation reported for patients with locally advanced disease a HR for survival 0.75, 95% CI 0.58-0.97 (p=0.03).[61,64] These would be the more relevant studies to justify a trial, where metformin use after diagnosis is investigated. Therefore, the profound difficulties with conducting pharmacoepidemiological work in this area mean that generally they should not be used for proof of concept assessment.

The effect of metformin, according to gender, and risk of PDAC was reported in a well-designed case-control study conducted in the United Kingdom, although this investigated etiology rather than treatment.[65] The authors documented a reduction in the risk of PDAC associated with those receiving long term metformin prescriptions in women (adjusted OR 0.43 (95% CI 0.23-0.80)) but not in men (adjusted OR 1.59 (95% CI 0.95-2.66)). The authors did not suggest a possible mechanism for this finding. In a univariate analysis, estrogens were not associated with risk of developing PDAC. In the 12 cohort studies discussed above, none reported a difference of effect of metformin, according to gender, and survival in PDAC. If a mechanism can be elucidated, evaluating responses to metformin in RCTs would be informative.

Proof of concept 3: Experimental medicine

Experimental medicine studies are those conducted in patients which may investigate tumor markers suggestive of a potential therapeutic benefit, rather than the primary outcome itself, such as survival. In a sub-group analysis, in the trial from the Netherlands, the *overall survival* was longer in patients with higher trough

concentrations of metformin (>1.0mg/L) after the first cycle of chemotherapy (median overall survival 9.1 months, vs 6.1 months, HR=0.37 (95%CI 0.14-0.98); log-rank p=0.049).[4] Also, patients in the metformin arm with a decrease in insulin concentrations had a longer *overall survival* than those without (median overall survival 18.6 months versus 5.7 months, HR=0.20 (95% CI 0.06-0.60), p=0.004). Importantly, the authors did not comment on if the cancer stages were similar in the two arms in these analyses. In patients who actively absorb metformin there may be a true survival benefit, although alternatively there may be significant patient selection biases. For example, patients with less aggressive and localized cancers, may be better able to ingest and absorb metformin than those with more advanced lesions. Therefore, a less advanced cancer stage, rather than the metformin itself may be associated with these improved survival times.

In the Italian trial, the authors investigated changes in baseline biomarkers in 35 of 60 (58%) patients who completed five cycles of treatment. After excluding patients with inadequate blood samples, 14 (48%) patients in the control arm and 12 (39%) patients in the metformin arm were included in the analysis. Patients who received metformin had a decrease in mean insulin of -11 pmol/L after cycle 5 compared to baseline (p=0.12), but in the control arm insulin increased by 7 pmol/L (p=0.22). The Italian trial also reported the frequency of the minor allele C of the ATM gene, namely 11% (CC), 54% (AC) and 35% (AA). In the metformin arm, patients with the C allele had the highest reduction in fasting plasma glucose after 1 month of treatment (CC -2.19 ±1.44 mmol/L (p=0.05), AC -0.94 ±1.46 mmol/L (p=0.05), AA -0.87 ±2.1 mmol/L (p=0.25)). No significant change in glucose was observed in the control arm.

A phase II trial registered in Canada (NCT02978547) is investigating the effects of neoadjuvant metformin on tumor cell proliferation and progression in resection

specimens. Patients receive metformin 500mg twice daily for a minimum of 7 days prior to surgery. Laboratory biomarkers (fasting glucose, insulin, GGT, HOMA index, plasma ctDNA and transcriptomic sequencing) will be assessed at baseline, before surgery and 2-3 months after surgery. This trial is due to start recruiting in 2019 and the results will add to the experimental medicine evidence. Other such work is required, particularly in patients with unresectable PDAC, for human experimental *proof of concept* information. Such work should consider changes in CA19-9 and other relevant clinical biomarkers, as well as genomic markers such as the C allele of the ATM gene.

Proof of concept 4: Metformin in other cancers

Further proof of concept data is if metformin is shown to be of benefit in clinical trials in other cancers. Metformin in the chemoprevention of colorectal cancer was investigated in a randomized phase 3 double-blind, placebo-controlled Japanese trial in preventing recurrent colorectal adenomas or hyperplastic polyps in 151 patients, without diabetes, who previously underwent polypectomy.[66] Patients in the treatment arm received 250mg metformin daily for one year. After one year, colonoscopies were repeated to assess the number and prevalence of recurrent adenomas or polyps. The prevalence of adenomas was significantly lower in the metformin group compared to the placebo (30.6 vs 51.6%) one (RR 0.60, 95% CI 0.39-0.92).

In post-menopausal women, 60 patients with hormone receptor positive locally advanced or metastatic breast cancer were randomly allocated an aromatase inhibitor (letrozole or exemestane) plus oral metformin 500mg twice daily (n=30) or placebo (n=30).[67] For 80% power, at least 30 patients in each arm were required to detect

an extra 1.38 months survival one year after diagnosis in median PFS. PFS was similar between the two groups, (median PFS 4.7 vs 6.0 months in the metformin vs placebo arms respectively (HR 1.2, 95% CI 0.7-2.1 p=0.48)). The median follow-up was 22.3 months. There are limitations with the interpretation of these null findings, as this trial was a small open-label phase II trial. Recruitment rate into the trial was not reported so generalizability could not be assessed.

There are currently 14 registered clinical trials that are actively following up, but no longer recruiting participants in other cancer sites namely: lung, gynecological, breast, prostate and hematological. There are 87 registered trials (ClinicalTrials.gov) in the recruitment stage of a trial, again across many cancer sites.

On-going trials of metformin in PDAC and feasibility work

Currently, there are several clinical trials in progress investigating metformin as an adjuvant or monotherapy in PDAC. Six are registered on ClinicalTrials.gov with 1 yet to commence recruitment, 3 currently recruiting, and 2 with follow-up but now not recruiting (table three). Three of these are in patients with resectable cancers and the remainder with metastatic cancer, although only 2 are assessing overall survival (death from any cause) as an outcome. The first is a double blinded, phase II RCT in China, currently recruiting 300 participants (NCT02005419). The patient population underwent curative resection, with adjuvant metformin prescribed for 28 days post-operatively. The second trial, in the United States, is investigating metformin as an adjuvant therapy in patients with metastatic disease (NCT01666730).

Conclusions

The role of metformin as an adjuvant chemotherapy in patients with PDAC remains unclear. However, there is substantial laboratory evidence for biological mechanisms

for anti-cancer effects of the drug. The interpretation of findings from pharmacoepidemiology and RCTs remains difficult due to limitations in their study design and conduct. Further large clinical trials, which address these limitations are required. However, before these are started, ideally further experimental medicine studies should be conducted to provide proof of concept data, and if so the feasibility of large RCTs first addressed.

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Table one: Cochrane risk of bias assessment tool [68]

***Trial 1: Metformin in patients with advanced pancreatic cancer: a double-blind, randomized, placebo-controlled phase 2 trial*. [4]**		
Bias	**Authors' judgement**	**Support for judgement**
Random sequence allocation (Selection bias)	*Low*	Hospital pharmacy staff randomly assigned patients in a 1:1 ratio by computer-generated permuted-block randomization (block size of six), to receive gemcitabine and erlotinib with either placebo or metformin. The allocation sequence was generated by the TENALEA Clinical Trial Data Management System (Amsterdam, Netherlands)
Allocation concealment (Selection bias)	*Low*	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 which was concealed by keeping block size confidential.
Blinding of participants and personnel (Performance bias)	*Low*	Patients, physicians, and study personnel were masked to treatment allocation.
Blinding of outcome assessment (Detection bias)	*Low*	The unmasked data became available to the investigators after the final database lock (July 22, 2014).
Incomplete outcome data (Attrition bias)	*High*	In the metformin arm, 58 participants discontinued (34 with tumor progression, 13 toxic effects, 3 deaths and 8 withdrew consent). In the placebo arm, 59 participants discontinued (42 had tumor progression, 8 toxic effects, 2 deaths and 7 withdrew consent).
Selective reporting (Reporting bias)	*Unclear*	The study protocol is available and endpoints in protocol matches published study outcome measures. One discrepancy is that the published trial states, "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." Published protocol states, "assessments on day 1 every cycle: metformin levels, biomarkers" There is no explanation for this protocol amendment.

Other bias	*High*	"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). 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)" Patients in placebo arm received more cycles of chemotherapy than those in the metformin group. Baseline CA19-9 levels were different between the two arms. As the sample size is small, randomization may not lead to equal distribution of characteristics between the two arms.
Trial 2: (Ir)relevance of Metformin Treatment in Patients with Metastatic Pancreatic Cancer: An Open-Label, Randomized Phase II Trial.[7]		
Random sequence allocation (Selection bias)	*Unclear*	"After signing the informed consent, patients were randomly allocated to receive the PEXG regimen with (arm A) or without (arm B) the addition of metformin." No further information reported regarding randomization.
Allocation concealment (Selection bias)	*Unclear*	"After signing the informed consent, patients were randomly allocated to receive the PEXG regimen with (arm A) or without (arm B) the addition of metformin." No further information reported regarding randomization.
Blinding of participants and personnel (Performance bias)	*High*	Open label (unblinding)
Blinding of outcome assessment (Detection bias)	*High*	Open label (unblinding)
Incomplete outcome data (Attrition bias)	*Low*	In the metformin arm, 17 (55%) participants discontinued (12 with radiological progression, 3 clinical progression, 1 toxic death and 1 withdrew consent). In the placebo arm, 14 (48%) participants discontinued (11 with radiological progression, 2 clinical progression and 1 medical decision). Therefore, withdrawal rates proportionate between the two groups were similar.
Selective reporting (Reporting bias)	*Low*	The study protocol is available, and the endpoints match published study outcome measures.
Other bias	*High*	The trial was powered assuming a baseline progression-free survival (PFS) of 50% at 6 months, and >70% for the metformin arm. This primary analysis required 78

	patients (39 per treatment arm) so the sample size was too small to detect this effect size. The significance level used was 10% i.e. a 10% chance of detecting a false positive result, which is above conventional full statistical significance.

Table two: Summary of pharmacoepidemiological studies for metformin and survival in PDAC

First author, year published, country	Study type	Total Participants	Exposure group	Reference group	Outcome measure(s) HR, 95% CI (p-value)
Chaiteerakij R, 2016, United States[61]	Retrospective cohort	980	Ever use of metformin (n=366) *Pre-diagnosis*	Never use of metformin (n=614)	0.92, 0.79-1.08 (p=0.30) Resectable cancers; 0.84, 0.62-1.15 (p=0.29) Locally advanced; 0.75, 0.58-0.97 (p=0.03)* Metastatic cancers; 1.19, 0.93-1.54 (p=0.17)
			Metformin exposure *after* PDAC diagnosis (n=85)		Metformin naïve at diagnosis; 1.04, 0.78-1.39 (p=0.77)
Currie CJ, 2012, United Kingdom[62]	Retrospective cohort	112,408 PDAC (n=2308)	Metformin use 90 days before diagnosis (n=2308)	Non-diabetic cohort (n=104016)	1.26, 0.85-1.85 (p=0.25)
			Metformin use ≤90 days immediately *after* diagnosis (n=1142)	Never use of metformin (n=2173)	0.65, 0.38-1.11 (p=0.12)
Kozak MM, 2016, United States[69]	Retrospective cohort	171	Ever use of metformin (n=18) Pre-*diagnosis*	Never use of metformin (n=153)	0.42, 0.30-0.94 (p=0.04)*
Lee SH, 2016, South Korea[70]	Retrospective cohort	237	Ever use of metformin (n=117) *Pre-diagnosis*	Never use of metformin (n=120)	0.61, 0.46-0.81 (p<0.001)*
Sadeghi N, 2012, United States[64]	Retrospective cohort	302	Ever use of metformin (n=117) *Pre-diagnosis*	Never use of metformin (n=185)	0.64, 0.48-0.86 (p=0.003)*

			Metformin use >2 years (n=26)	Metformin use <2 years (n=47)	Metformin use (yrs) 2-5; 0.51, 0.28-1.05 (p=0.07) >5; 0.82, 0.40-1.69 (p=0.59)
Choi Y, 2015, Korea[71]	Retrospective cohort	349 (T2DM n=183)	Ever use of metformin in T2DM (n=56) Ever use of metformin in full cohort *Pre-diagnosis*	Never use of metformin	0.69, 0.49-0.98 (p=0.04)* 0.70, 0.49-0.99 (p=0.04)*
Hwang AL, 2013, United Kingdom[72]	Retrospective cohort	516	Ever use of metformin (n=247) *Pre-diagnosis*	Never use of metformin (n=269)	1.11, 0.89-1.38 (p=0.37)
Jo A, 2015, South Korea[73]	Retrospective cohort	764	Ever use of metformin (n=530) *Pre-diagnosis*	Never use of metformin (n=234)	0.73, 0.61-0.87 (p<0.001)*
Frouws MA, 2017, The Netherlands[63]	Retrospective cohort	907	Ever use of metformin (n=77) *Pre-diagnosis*	Never use of metformin (n=830) Sulfonylurea use (n=43)	Adjusted OR, 95% CI 0.86, 0.66-1.12 (p=0.26) Adjusted OR, 95% CI 0.86, 0.50-1.46 (p=0.57)
Amin S, 2016, United States[74]	Retrospective cohort	1916	Ever use of metformin (n=1098) *Pre-diagnosis*	Never use of metformin (n=818)	0.88, 0.81-0.96 (p<0.01)*
Cerullo M, 2016, United States[60]	Retrospective cohort	3393	Ever use of metformin (n=456) *Pre-diagnosis* Metformin dose <1000mg/day (n=254) Metformin dose ≥1000mg/day (n=173)	Never use of metformin (n=2937)	0.79, 0.57-0.93 (p<0.005)* 0.80, 0.65-0.98 (p=0.03)* 0.70, 0.53-0.92 (p=0.01)*
Ambe CM, 2016, United States[75]	Prospective cohort	44	Ever use of metformin (n=19) *Pre-diagnosis*	Never use of metformin (n=25)	0.70, 0.31-1.59 (p=0.39)

* indicates statistical significance

Table three: Summary of trials registered on ClinicalTrials.gov

Title	Total participants	Randomization	Treatment arm	Primary outcome	Status	End date
Resectable PDAC						
A Pilot Trial of Stereotactic Body Radiation Therapy and Metformin for Borderline-Resectable and Locally-Advanced Pancreatic Adenocarcinomas (NCT02153450)	15	No	Metformin PO daily or BID on days -11 to -1. Stereotactic radiosurgery 5 days a week for 5 weeks and receive concurrent metformin PO BID for 5 weeks. Laparotomy on week 6 (or weeks 5-7).	Dose-limiting toxicity (DLT) rate	Recruiting	Oct 2018
A Phase II, Randomized, Double-blind, Placebo Controlled Study to Evaluate the Efficacy and Safety of the Combination of Gemcitabine and Metformin in Treating Patients With Pancreatic Cancer After Curative Resection (NCT02005419)	300	Yes	Gemcitabine at 1000 mg/m^2 on days 1, 8, and 15; metformin at 2 g on days 1-28	Recurrence-free survival	Recruiting	June 2017
The Effects of Neoadjuvant Metformin on Tumor Cell Proliferation and Tumor Progression in Pancreatic Ductal Adenocarcinoma (NCT02978547)	20	No	Metformin 500 mg PO BID for at least 7 days, until 2 days prior to surgery.	Tumor cell proliferation	Not yet recruiting	Jan 2021
Unresectable PDAC						
A Pilot Study of Gemcitabine, Abraxane, Metformin and a Standardized Dietary Supplement (DS) in Patients With Unresectable Pancreatic Cancer (NCT02336087)	21	No	Gemcitabine and paclitaxel albumin-stabilized nanoparticle formulation IV on days 1, 8, and 15. Metformin PO BID day -6 and dietary supplement PO BID day -3. Repeat every 28 days	Compliance, toxicity and feasibility	Recruiting	Sept 2018

A Phase II Study of Metformin Plus Modified FOLFOX 6 in Patients With Metastatic Pancreatic Cancer (NCT01666730)	50	No	Metformin PO BID on days 1-14 and FOLFOX therapy comprising leucovorin, fluorouracil and oxaliplatin IV on day 1. Repeat every 14 days	Median overall survival	Active, not recruiting	Mar 2018
An Exploratory Study of Metformin With or Without Rapamycin as Maintenance Therapy After Induction Chemotherapy in Subjects With Metastatic Pancreatic Adenocarcinoma (NCT02048384)	22	Yes	Metformin + rapamycin Arm B patients will receive 850mg orally twice a day and rapamycin 4mg orally once a day on a 28-day cycle.	Safety and feasibility	Active, not recruiting	Dec 2019

Table four: Summary of findings of the review

Proof of concept 1: Biological plausibility of metformin as an adjunctive chemotherapeutic drug demonstrated in laboratory studies
• The potential anti-cancer effects of metformin are through AMPK activation and reduction of circulating insulins levels, which in excess are mitogenic.
Proof of concept 2: Pharmacoepidemiological evidence
• Interpretation of findings from pharmacoepidemiological studies in PDAC should be cautious given biases. • To date, most cohort studies have demonstrated a positive association with metformin on survival in PDAC, in particular those with locally advanced disease.
Proof of concept 3: Experimental medicine
• In the Netherlands trial, overall survival was longer in patients with higher trough concentrations of metformin and in those with decreased insulin concentrations.
Proof of concept 4: Metformin in other cancers
• Metformin has a chemoprevention role in colorectal cancer in preventing colorectal adenoma recurrence. • There are many trials underway in other cancers.
Future work required
• In order to inform and justify a future large RCT, further experimental medicine studies are required. • Such trials would need to investigate the significance of PPIs, genetic variants and degree of hepatic metastases.

Figure One: Potential anti-cancer effects of metformin. Metformin inhibits complex 1 of the mitochondria and reduces ATP production. A reduction of ATP consequently activates AMPK and inhibits processes involved in cell growth and cell division. ⊥, inhibitor arrow. ↑, activator arrow. LKB1, Liver kinase B1; AMPK, AMP-activated protein kinase; TSC, Tuberous sclerosis complex; mTOR, Mechanistic target of rapamycin.

Figure One