

Repurposing metformin for the treatment of gastrointestinal cancer

Ademar Dantas Cunha Júnior, Arinilda Campos Bragagnoli, Felipe Osório Costa, José Barreto Campello Carvalheira

ORCID number: Ademar Dantas Cunha Júnior [0000-0001-8112-4766](https://orcid.org/0000-0001-8112-4766); Arinilda Campos Bragagnoli [0000-0002-5165-0797](https://orcid.org/0000-0002-5165-0797); Felipe Osório Costa [0000-0002-1997-8389](https://orcid.org/0000-0002-1997-8389); José Barreto Campello Carvalheira [0000-0002-0136-0943](https://orcid.org/0000-0002-0136-0943).

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Ademar Dantas Cunha Júnior, Felipe Osório Costa, José Barreto Campello Carvalheira, Department of Internal Medicine, Division of Oncology, University of Campinas (UNICAMP), Campinas 13083-970, São Paulo, Brazil

Arinilda Campos Bragagnoli, Division of Oncology, Hospital de Câncer de Barretos, Barretos 14784-400, São Paulo, Brazil

Corresponding author: José Barreto Campello Carvalheira, MD, PhD, Associate Professor, Department of Internal Medicine, Division of Oncology, University of Campinas (UNICAMP), Tessália Vieira Camargo, S/N, Campinas 13083-970, São Paulo, Brazil. jbcc@unicamp.br

Abstract

Diabetes mellitus type 2 and cancer share many risk factors. The pleiotropic insulin-dependent and insulin-independent effects of metformin might inhibit pathways that are frequently amplified in neoplastic tissue. Particularly, modulation of inflammation, metabolism, and cell cycle arrest are potential therapeutic cancer targets utilized by metformin to boost the anti-cancer effects of chemotherapy. Studies *in vitro* and *in vivo* models have demonstrated the potential of metformin as a chemo- and radiosensitizer, besides its chemopreventive and direct therapeutic activity in digestive system (DS) tumors. Hence, these aspects have been considered in many cancer clinical trials. Case-control and cohort studies and associated meta-analyses have evaluated DS cancer risk and metformin usage, especially in colorectal cancer, pancreatic cancer, and hepatocellular carcinoma. Most clinical studies have demonstrated the protective role of metformin in the risk for DS cancers and survival rates. On the other hand, the ability of metformin to enhance the actions of chemotherapy for gastric and biliary cancers is yet to be investigated. This article reviews the current findings on the anti-cancer mechanisms of metformin and its apparatus from pre-clinical and ongoing studies in DS malignancies.

Key Words: Antidiabetic treatments; Gastrointestinal tumors; Therapeutic target

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Core Tip: Modulation of cell function into the neoplastic and around the microenvironment tissue are possible cancer targets utilized by metformin to raise

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chemotherapy's anti-tumor outcomes. Herein we review the studies that have demonstrated the likelihood of metformin as chemo and radiosensitizer, in addition to its chemopre-ventive and direct therapeutic activity in gastrointestinal tumors.

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INTRODUCTION

Diabetes mellitus type 2 (DM2) and cancer share several risk factors[1]. Notably, obesity and metabolic syndrome, with their inherent biological connections, such as hyperinsulinemia[2] and chronic inflammation[3]. Furthermore, some antihyperglycemic medications (*e.g.*, sulfonylureas and insulin) used for the treatment of DM2 may increase cancer risk[4]. Particularly, central obesity, physical inactivity, and perhaps a low dietetic polyunsaturated fat to saturated fat ratio are major risk factors for insulin resistance and hyperinsulinemia and seem to be related to cancer risk[5]. All of them have been recognized as proposed gears holding those relationships[6-9]. Epidemiologic studies and meta-analyses have suggested that patients with DM2 have a higher incidence and mortality from malignancies[10,11], including digestive system (DS) cancers[12-15].

Metformin is a well-known oral hypoglycemic drug that belongs to the biguanide class and has been used to treat DM2 for almost a century[16]. Importantly, those patients with DM2 with long-term use of metformin have a decreased tumor incidence and lower cancer-associated mortality[17-21]. Furthermore, recent research indicates that metformin can have direct anti-cancer activity against many tumor cells, including tumor stem cells[22,23], therefore, carrying out pleiotropic effects in both the cancer cell and the neoplastic microenvironment[24]. Their potential mechanisms are insulin-dependent [*via* insulin growth factor (IGF) receptor, phosphatidylinositol 3 kinase (PI3K), and Akt/mammalian target of rapamycin (mTOR)][25,26] and insulin-independent [*via* adenosine kinase monophosphate (AMPK), tuberous sclerosis complex (TSC), and mTOR][27,28]. Moreover, it promotes antitumor immunity-related metabolic checkpoints in T-cells, cancer cells, as well as associated with immunosuppressive cells of the tumor milieu[29]. Furthermore, it might interfere with the gut microbiota and have systemic impacts on body metabolism[30,31]. This article aims to review the rationale of metformin as a drug that might be repurposed for DS cancer treatment.

MECHANISM OF ACTION OF METFORMIN AS AN ANTI-CANCER AGENT

Two potential mechanisms for the antineoplastic action of metformin have been suggested (Figure 1). First, metformin can directly activate AMPK, resulting in inhibition of downstream Akt/mTOR signaling and consequent suppression of cell proliferation[32,33]. Second, metformin-induced reductions in circulating insulin and IGF concentrations may reduce activation of the IGF receptor signaling axis, resulting in decreased growth promotion and mitogenesis[2,34]. Hence, the anti-cancer effects of metformin are mediated through a systemic improvement in the metabolic milieu or directly on tumor cells[35].

The noticeable intracellular metabolic change caused by metformin is the decreased accumulation of glycolytic intermediates and a coordinated decrease in tricarboxylic acid (TCA) cycle intermediates[36,37]. Moreover, the activation of AMPK reduces fatty acid synthase (FAS) gene expression in the synthesis of fatty acids[32]. Furthermore, metformin offers other direct anti-tumor effects by (1) decreasing specific protein (Sp) transcription factors and Sp-related oncogenic proteins[38,39]; (2) decreasing AMPK-dependent c-Myc oncogene; (3) increasing other miRNAs, such as mir33a[40]; (4) increasing other miRNAs, such as miR-26a[41]; (5) reducing endogenous reactive oxygen species and associated DNA damage[42]; (6) reducing Sonic hedgehog

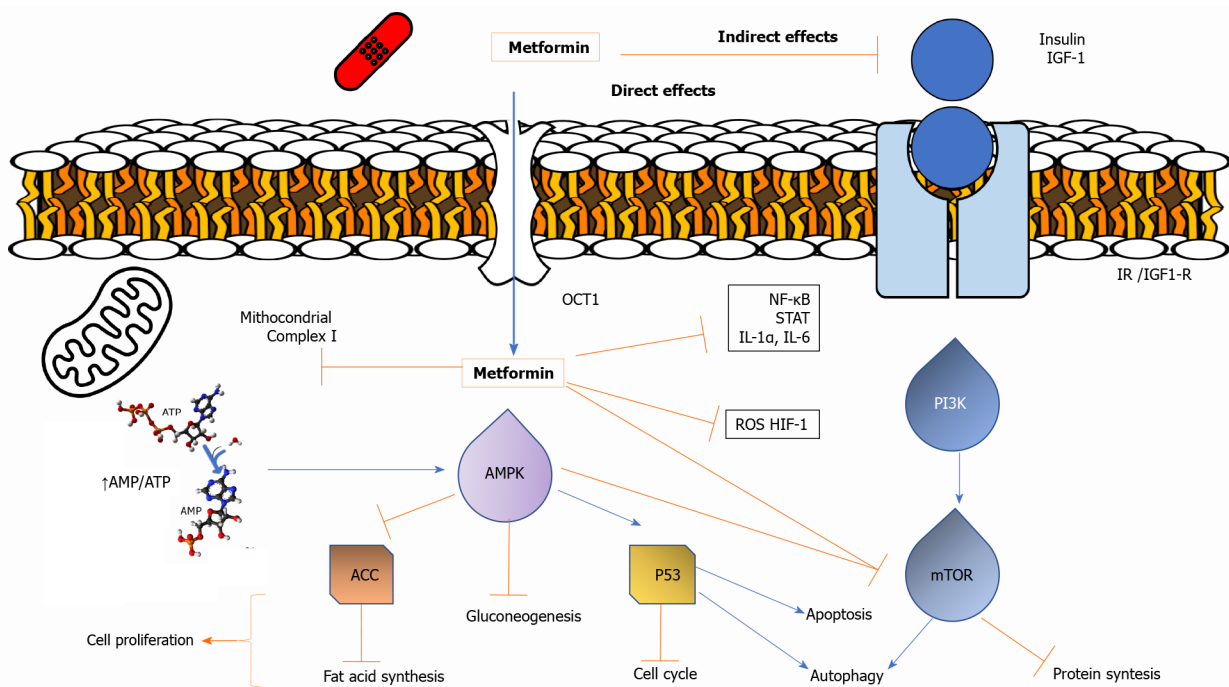


Figure 1 Overview of cellular mechanisms of metformin in cancer. Metformin inhibits mitochondria complex I, stimulates the adenosine monophosphate-activated protein kinase signaling pathway, and/or inhibits the insulin signaling pathway. Blue lines represent activated pathways while red lines represent inhibitory pathways. AMPK: Adenosine monophosphate-activated protein kinase; ACC: Acetyl-CoA carboxylase; HIF-1 α : Hypoxia-inducible factor-1 alpha; IGF: Insulin growth factor; IGF-1: Insulin-like growth factor-1; IGF-1R: Insulin-like growth factor-1 receptor; IR: Insulin receptor; IL-1: Interleukin 1; IL-6: Interleukin-6; NF- κ B: Nuclear factor kappa; OCT1: Organic cation transporter 1; ROS: Reactive oxygen species; STAT: Signal transducer and activator of transcription; AMP: Adenosine monophosphate; ATP: Adenosine triphosphate; PI3K: Phosphoinositide 3-kinase; mTOR: Mechanistic target of rapamycin.

expression[43]; (7) reducing expression of angiogenic factor CCN1, which inhibits invasion induced by the stromal cell-derived factor-1 and reducing levels of type 4 chemokine receptor[44]; and (8) inhibiting Rac1 GTPase activity[45]. Finally, metformin might interfere with the gut microbiota[30,31], as well as interfere with the balance between T-cells and associated immunosuppressive cells in the tumor milieu[29].

Insulin-dependent or indirect effects

A central signal transduction pathway involved in cancer is the PI3K/Akt/mTOR pathway, which, when hyperactivated, leads to deregulation of survival and cell growth[28,46,47]. IGF-1 is a more potent mitogen than insulin and, like insulin, binds to its particular growth factor receptor and stimulates cell growth and anti-apoptotic activity *via* MAPK/ERK or Ras/Raf/MEK/ERK and PI3K/Akt/mTOR signaling [2,34]. In addition, IGF-1 inhibits PTEN, a phosphatase that deactivates PI3K/Akt/mTOR[2]. The indirect mechanisms of metformin action include inhibition of hepatic gluconeogenesis and stimulation of peripheral glucose absorption, which ultimately lead to decreased blood glucose and insulin levels. Thus, the most apparent mechanism of insulin-dependent metformin involves decreasing insulin levels, which reduces insulin binding to the insulin receptor (IR), inhibiting tumor growth[48]. A reduction of insulin/IGF-1 levels is, at least in part, involved in the antiproliferative activity of metformin[49]. Additionally, metformin downregulates IGF-R and IR by decreasing the promoter activity of receptor genes with subsequent Akt/mTOR and MAPK/ERK signaling inhibition[50,51].

Insulin-independent or direct effects

Metformin activates AMPK by inhibiting mitochondrial complex I, which leads to impaired mitochondrial function, decreased adenosine triphosphate synthesis, increased adenosine monophosphate, and subsequent phosphorylation and activation by LKB1[52]. Activated AMPK then phosphorylates TSC2, which negatively regulates mTOR activity[53]. Activation of LKB1 and AMPK, AMPK-induced stabilization of TSC1-TSC2 (inhibitor of Rheb, an mTORC1 activator), and activation of the tumor suppressor p53[54]. Moreover, independent of AMPK, metformin impedes mTORC1 by raising p53-dependent expression of REDD1 and repressing Rags[55]. Metformin

also retards transformation by inhibiting mediators of the inflammatory response, including transcription factors (nuclear factor kappa, Signal transducer and activator of transcription 3, and Forkhead box O signaling), and downregulating Lin28B, most Let-7 miRNA family members, and inflammatory molecules [interleukin (IL)-1 α , IL-1 β , IL-6, and vascular endothelial growth factor (VEGF)][56].

Metformin has other AMPK-mediated actions that may be implicated in cancer. Through the activation of AMPK, metformin causes the suppression of FAS gene expression, which is involved in the synthesis of fatty acids, resulting in reduced lipogenesis, increased fatty acid oxidation, and decreased cell proliferation[32,57]. The activation of AMPK also modulates cyclin D1 (cell cycle protein), p21 and p27 (cyclin-dependent protein kinase), which further contribute to its anti-cancer effects[55,58]. Interestingly, metformin may act as a chemosensitizer, for example, increasing the 5-fluorouracil (5-FU) and paclitaxel sensitivities of cancer cell lines[59,60]. The ability of metformin to disconnect the electron transport chain by inhibiting complex I (NADH dehydrogenase) strongly induces cell death when glucose is limited. Metformin also reduces the hypoxic activation of hypoxia inducing factor (HIF-1), suggesting that the effects of metformin are increased in hypoglycemic and hypoxic conditions[61].

Other mechanisms

As a drug that controls metabolism, metformin promotes a coordinated decrease of TCA cycle intermediates, including succinate, fumarate, malate, citrate, and α -ketoglutarate[36,37]. The dependency of neoplastic cells on glutamine metabolism has been shown to be reprogrammed by the Kras oncogenic pathway through a single pathway involving serum glutamic-oxaloacetic transaminase, which maintains the cellular redox states essential to mitochondria and offers innovative therapeutic targets in combination with metformin[62].

Metformin can exert antitumor activity by increasing CD8⁺ T-cells[63,64]. It might inhibit apoptosis of CD8⁺ tumor infiltrating lymphocytes and prevent immune exhaustion[63,65]. Furthermore, metformin might adjust the expression profile of immune checkpoints[66], such as programmed death ligand 1, in the context of the neoplasm[37], thereby suggesting that a combination of metformin might have the potential to enhance the strength of cancer immunotherapy[63].

There is evidence that epigenomic modifications by metformin may contribute to its anti-cancer properties[67]. Metformin might regulate the activity of numerous epigenetic modifying enzymes, principally by modulating the activation of AMPK. Activated AMPK can phosphorylate several substrates, comprising epigenetic enzymes, such as histone acetyltransferases (HATs), class II histone deacetylases (HDACs) and DNA methyltransferases (DNMTs), usually resulting in their inhibition; however, HAT1 activity may be increased. Metformin has also been related to the diminished expression of various histone methyltransferases[68], enhancing the activity of the class III HDAC SIRT1 and minimizing the influence of DNMT inhibitors[69,70].

METFORMIN STUDIES IN DIGESTIVE SYSTEM MALIGNANCIES

Metformin in colorectal cancer

Cell lines and animal models: Metformin promotes cell cycle arrest in the G0/G1 phase in colorectal cancer (CRC) cell lines. It also decreases the expression of c-Myc and causes down-regulation of IGF-1R[71]. Consequently, up-regulation of the adenosine A1 receptor induces apoptosis[72]. Additionally, it was shown that metformin enhances the activity of the *Sprouty2* gene, which suppresses colon cancer growth[73].

The combination of metformin with 5-FU was investigated on the SW620 CRC cell line and on patients with DM2. The study showed that metformin plus 5-FU treatment significantly inhibited the proliferation of SW620 cells compared with that in monotherapy. Additionally, the examination of 86 CRC tissue samples obtained from patients with DM2 revealed that treatment with metformin decreased the proportion of poorly differentiated tumors[74]. Moreover, a synergistic effect of 5-FU and metformin was observed in a 5-FU resistant cell line[74] and metformin radiosensitizer CRC cells, with reduced survival of ionization-resistant cells[75]. Consistently, the association of oxaliplatin, 5-FU and metformin also demonstrated a superior anti-tumor activity in chemoresistant HT-29, and HCT-116 cells compared to that with the drugs separately[76].

In 1977, it was firstly reported that phenformin inhibits metabolic immunosuppression in rats[77]. Since then, several reports have demonstrated that metformin has both chemopreventive and therapeutic activities in animal models of CRC. For instance, metformin treated ApcMin/+ mice showed significantly smaller polyps[78], and carcinogen-induced animal models that received metformin had a reduction of aberrant crypt foci[79], indicating the chemopreventive effect of metformin. Moreover, the association of metformin with D3 vitamin demonstrated a chemopreventive effect against 1,2-dimethylhydrazine (DMH)-induced CRC in rats and DMH-dextran sodium sulfate-induced colitis-associated CRC in mice[80]. On the other hand, treatment with metformin, 5-FU and oxaliplatin demonstrated superior antiproliferative effects in SCID mice bearing CRC[76]. In avatar models, metformin suppressed the tumor growth in the patient-derived xenografts by 50%[81]. In the same study, when metformin was combined with 5-FU, the tumor growth was inhibited up to 85%[81].

Clinical use of metformin

Metformin and CRC risk: As shown in Table 1 many case-control and cohort studies and associated meta-analyses have evaluated DS cancer risk and metformin use. Specifically, a decreased risk of CRC was found in the majority of studies[82-86], but no association or an increased risk of CRC was found in some of them[87-91]. Although these different results may be related to biases, a large cohort study that used adequate methods to minimize biases also concluded that metformin use decreased the risk of CRC[92].

Saliently, Cardel *et al*[82] demonstrated, in a case-control study, that the risk of CRC was decreased by 17% (OR: 0.83, 95%CI: 0.74-0.92) among patients treated with metformin compared to that among patients not using metformin[82], while Liu *et al*[93] showed a 22% risk reduction for the development of CRC[93]. Importantly, the role of metformin for CRC prophylaxis was addressed in a prospective Japanese phase III trial that demonstrated that low metformin doses for 1-year reduced polyp formation and colorectal adenomas in non-diabetic patients at high risk for new polyps[94]. However, further studies are necessary to draw a definitive conclusion.

Metformin and CRC treatment

Table 2 summarizes the clinical studies of metformin on DS cancers treatment. Specifically related to CRC, a Korean study of 595 patients with diabetes who had CRC with clinical stages I to IV showed that patients using metformin had higher overall survival (OS) and specific cancer survival compared to patients who did not use it[95]. In accordance, metformin use in 424 diabetic patients with CRC was associated with an OS of 76.9 mo *vs* 56.9 mo in patients not using metformin ($P = 0.048$)[95]. After adjusting for possible confounding factors, the study showed that patients with DM2 treated with metformin had a 30% increase in OS when compared to patients with DM2 treated with other antidiabetic drugs[95]. Recent meta-analyses demonstrated that metformin increases the OS of patients with CRC, as well as a 10% reduction in the incidence of the disease[96,97]. The ASAMET trial, an ongoing randomized, phase II, double-blind, placebo-controlled trial aims to determine the effect of low-dose aspirin and metformin in patients with stage I-III CRC in reducing CRC mortality rates and adenoma recurrence[98]. The 160 patients with CRC were divided in four arms: aspirin, metformin, aspirin plus metformin and placebo for a duration of 1 year.

The radiotherapy-induced tumor response was improved with metformin in a Korean retrospective study that evaluated patients with localized rectal cancer. The diabetic patients receiving metformin had significantly more tumor regression grade 3-4 ($P = 0.029$) and higher lymph node downstaging ($P = 0.006$) as compared to patients not receiving the medication. However, the disease-free survival (DFS) and OS was not affected[99]. Consistently, a study with 482 patients examined the effect of metformin use on pathologic complete response (pCR) rates and outcomes in patients submitted to neoadjuvant chemoradiotherapy for rectal cancer. The pCR rates were higher in patients with DM2 taking metformin (35%) compared with those in nondiabetic patients (16.6%) and patients with DM2 not using metformin (7.5%). Additionally, significantly increased DFS and OS was found in patients taking metformin[100].

A phase II clinical trial addressed the combination of metformin with 5-FU in patients with refractory CRC. It demonstrated a disease control rate in 8 wk of 22%, with a median OS of 7.9 mo and progression-free survival (PFS) of 1.8 mo[101]. A trial of our group with a similar design that analyzed the combination of irinotecan with metformin found 41% disease control rate and OS of 8.2 mo[102]. Further randomized prospective studies are needed to establish metformin as a modern drug for the treatment of refractory CRC.

Table 1 Selected clinical studies of metformin on digestive system cancers chemoprevention

Ref.	Study design and population	Inclusion criteria	Combined interventions /drugs	Main findings
			Comparison groups	Risk estimates and 95%CI
Colorectal cancer				
Cardel <i>et al</i> [82], 2014	Case-control study. Cases-controls: 2088:9060	Cases: DM2 with CRC. Controls: DM2 without CRC	Metformin user <i>vs</i> nonuser	OR: 0.83 (0.68-1.00)
Lee <i>et al</i> [83], 2011	Prospective Cohort, Taiwan. <i>n</i> = 480984	DM2 and cancer free subjects	Metformin user <i>vs</i> nonuser	HR: 0.36 (0.13-0.98)
Sehdev <i>et al</i> [84], 2015	Case control study. Cases-controls: 2682:5365	Cases: DM2 with CRC. Controls: DM2 without CRC	Metformin user <i>vs</i> nonuser	OR: 0.85 (0.76-0.95)
Tseng <i>et al</i> [84], 2012	Retrospective Cohort. Men: 493704. Women: 502139	Subjects covered by National Health Insurance without CRC	Metformin user <i>vs</i> nonuser	RR: 0.64 (0.49-0.84)
Zhang <i>et al</i> [86], 2011	Meta-analysis. 108161 DM2 patients	Studies conducted in humans that evaluate metformin and CRC	Metformin user <i>vs</i> nonuser	RR: 0.63 (0.47-0.84)
Kowall <i>et al</i> [87], 2015	Retrospective Cohort, United Kingdom. 80263 DM2 patients	Patients aged 30-89 years with DM2 diagnosis	Metformin user <i>vs</i> sulfonylurea user	HR: 1.04 (0.82-1.31)
Lin <i>et al</i> [88], 2015	Prospective Cohort. 36270 DM2 patients. 145080 non DM2	Patients older than 20 years old DM2 and Cancer- free	Metformin user <i>vs</i> nonuser	HR: 0.74 (0.53-1.03)
Smiechowski <i>et al</i> [89], 2013	Case-control, United Kingdom. Cases-controls: 607:5837	DM patients treated with non-insulin antidiabetic agents	Metformin user <i>vs</i> nonuser	RR: 0.93 (0.73-1.18)
Bodmer <i>et al</i> [90], 2012	Case control, United Kingdom. Cases-controls: 920:5519	Cases: DM2 with CRC. Controls: DM2 without CRC	Metformin user <i>vs</i> nonuser	Men: OR: 1.81 (1.25-2.62). Women: OR: 1.00 (0.63-1.58)
Knapen <i>et al</i> [91], 2013	Retrospective Cohort, Denmark. 177281 DM2 with OHA	Oral antidiabetic drug users were matched 1:3 with population-based reference group	Biguanide user <i>vs</i> non-diabetic	HR: 1.19 (1.08-1.30)
Bradley <i>et al</i> [92], 2018	Retrospective Cohort, Northern California. 47351 DM2 patients	DM2 and no history of cancer or metformin use	Long-term metformin use (5 years) <i>vs</i> nonuser	All population: HR: 0.78 (0.60-1.02). Men: HR: 0.65 (0.45-0.94)
Liu <i>et al</i> [175], 2017	Meta-analysis. 20 case-control and cohort studies	Studies about metformin therapy and risk of adenoma/CRC in DM2 patients	Metformin user <i>vs</i> nonuser	Adenoma: OR: 0.75 (0.59-0.97). Carcinoma: OR: 0.781 (0.7-0.87)
Higurashi <i>et al</i> [94], 2016	RCT, phase 3. <i>n</i> = 151 patients with resected adenomas or polyps	Non- diabetic adult patients who had previously had single or multiple colorectal adenomas or polyps resected by endoscopy	Metformin 250 daily or placebo (1:1) for 1 yr	Adenoma: RR 0.60 (0.39-0.92)
Gastric cancer				
Tseng <i>et al</i> [107], 2016	Retrospective Cohort, Taiwan. 287971 DM2 with metformin. 16217 DM2 without metformin	DM2 patients newly treated with antidiabetic drugs	Metformin user <i>vs</i> nonuser	HR: 0.45 (0.36-0.56)
Dulskas <i>et al</i> [108], 2020	Retrospective Cohort study. <i>n</i> = 99992	DM2 patients with gastric cancer	Metformin user <i>vs</i> nonuser	SIR: 0.75 (0.66-0.86)
Ruiter <i>et al</i> [109], 2012	Retrospective Cohort study. 85289 DM2 patients	DM2 with more than one prescription of antidiabetic drugs	Metformin user <i>vs</i> sulfonylurea user	HR: 0.90 (0.88-0.91)
Kim <i>et al</i> [110], 2014	Retrospective cohort study. 39978 DM2 patients	DM2 receiving oral antidiabetic drugs	Metformin user and non-insulin user <i>vs</i> nonuser	HR: 0.73 (0.53-1.01)
Cheung <i>et al</i> [112], 2019	Prospective Cohort study. 7266 DM2	DM2 with prescription of therapy for H. pylori. Exclusion: history of GC	Metformin user <i>vs</i> nonuser	HR: 0.49 (0.24-0.98)
Tsilidis <i>et al</i> [113], 2014	Retrospective Cohort study. 51484 metformin. 18264 sulfonylureas	DM2 receiving oral antidiabetic drugs	Metformin user <i>vs</i> sulfonylurea user	HR: 0.96 (0.60-1.56)
de Jong <i>et al</i> [114], 2017	Retrospective Cohort study, Netherlands. 57621 DM2 with OHA	DM2 receiving oral antidiabetic drugs	Metformin user <i>vs</i> nonuser	HR: 0.97 (0.82-1.15)

Zheng <i>et al</i> [115], 2019	Prospective Cohort study. 544130 DM2 patients	Diabetes Cohort: DM2 receiving antidiabetic drugs. Matched cohort: common-medication users. Exclusion: History of GC or gastrectomy	Metformin user <i>vs</i> nonuser	Non-cardia: HR: 0.93 (0.78-1.12). Cardia: HR: 1.49 (1.09-2.02)
Shuai <i>et al</i> [116], 2020	Meta-analysis. 11 cohort studies	Studies conducted in humans that evaluate metformin and GC risk	Metformin user <i>vs</i> nonuser	HR: 0.79 (0.62-1.00)
Zhou <i>et al</i> [117], 2017	Meta-analysis. 7 Cohort studies. <i>n</i> = 591077	Studies conducted in humans that evaluate metformin and GC risk	Metformin user <i>vs</i> nonuser	HR: 0.76 (0.64-0.91)
Pancreatic ductal adenocarcinoma				
Currie <i>et al</i> [123], 2009	Retrospective cohort study. <i>n</i> = 62.809 DM2. Comparison between treatment: Metformin alone; Sulfonylurea alone; metformin plus sulfonylurea; insulin	DM2 developed > 40 years of age; United Kingdom residents	Metformin <i>vs</i> Sulfonylurea. Metformin <i>vs</i> Insulin	HR: 0.20 (0.11-0.36). HR: 0.22 (0.12-0.38)
Li <i>et al</i> [124], 2009	Hospital-based case control. Cases-controls: 973:863. Comparison between treatment: Metformin; insulin secretagogues; Other antidiabetic medications; insulin	DM subjects; cases: Newly PDAC diagnosed. Controls: Nonblood relative controls; United States residents	Metformin user <i>vs</i> nonuser	OR: 0.38 (0.22-0.69)
Soranna <i>et al</i> [126], 2012	Meta-analysis of 17 case-control and cohort studies. Any cancer: 17 case-control and cohort studies; 37632 cases. PDAC: 4 case-controls and retrospective cohort studies; 1192 cases	DM2 patients exposed to metformin alone or combined to sulfonylurea	Metformin user <i>vs</i> nonuser	RR: 0.38 (0.14-0.91)
Zhang <i>et al</i> [125], 2013	Meta-analysis of 37 case-control and cohort studies. <i>n</i> = 1535636	DM2 patients on treatment	Metformin user <i>vs</i> nonuser	SRR: 0.54 (0.35-0.83)
Hepatocellular carcinoma				
Donadon <i>et al</i> [159], 2010	Clinic-hospitalbased case control. Cases-controls: 190:359	Cases: HCC patients. Controls: Liver cirrhosis patients and healthy controls	Metformin <i>vs</i> sulfonylurea. Metformin <i>vs</i> insulin	OR: 0.39 (0.22-0.73). OR: 0.21 (0.11-0.42)
Hassan <i>et al</i> [158], 2010	Hospital-based case control. Cases-controls: 122:86	Cases: HCC. Controls: Healthy controls	Metformin user <i>vs</i> nonuser	OR: 0.30 (0.20-0.60)
Ma <i>et al</i> [160], 2017	Meta-analysis of 19 case-control and cohort studies and post hoc analysis of RCT of DM2 patients. <i>n</i> = 550.882	DM2 exposed to metformin or biguanide	Metformin user <i>vs</i> nonuser	OR: 0.52 (0.40-0.68)
Intrahepatic cholangiocarcinoma				
Chaiteerakij <i>et al</i> [168], 2013	Clinic-hospital based case-control. Cases-controls: 612:594	Cases: ICC patients. Controls: Non-cancer patients	Metformin user <i>vs</i> nonuser	OR: 0.40 (0.20-0.90)

PDAC: Pancreatic ductal adenocarcinoma; HR: Hazard ratio; OHA: Oxidized hyaluronate; RCT: Randomized clinical trial; HCC: Hepatocellular carcinoma; ICC: Intrahepatic cholangiocarcinoma; CRC: Colorectal cancer.

Interestingly, a randomized trial that included 40 patients with stage III CRC evaluated the use of metformin in preventing oxaliplatin-induced neuropathy. After the 12th cycle of the FOLFOX-4 regimen, in the metformin group, there were fewer patients with grade 2 and 3 neuropathy as compared to the control arm (60% *vs* 95%, *P* = 0.009). Moreover, significantly higher total scores on the Ntx-12 questionnaire and pain score were found in the metformin arm. The serum levels of neurotensin and malondialdehyde were also significantly lower in the metformin arm after 6 and 12 cycles[103].

Furthermore, there are ongoing trials evaluating the role of metformin in CRC. We highlight, in adjuvant setting, a phase 3 trial (NCT02614339) with high-risk stage II and stage III CRC that aims to evaluate the impact of metformin for 48 mo on disease free survival. In refractory CRC setting, there is an interesting phase 2 trial is recruiting patients to explore the combination of the immune checkpoint inhibitors, such as nivolumab and metformin (NCT03800602).

Metformin and gastric cancer

The effect of metformin alone or in combination with cisplatin or rapamycin was studied in a tumor xenograft model[104]. It demonstrated that metformin alone decreased tumor volume. The combination of metformin with cisplatin, rapamycin or both increase the effect of each drug alone and inhibited the peritoneal dissemination of gastric cancer (GC)[104]. In accordance, Wu performed an *in vitro* study with AGS cell lines that analyzed how the association of metformin with cisplatin or adriamycin or paclitaxel enhanced the effects of each drug alone[105]. In striking contrast, Lesan

Table 2 Selected clinical studies of metformin on digestive system cancers treatment

Ref.	Study design and population	Inclusion criteria	Combined interventions /drugs	Main findings
Colorectal cancer				
Ramjeesingh <i>et al</i> [99], 2016	Retrospective cohort. 1394 all stages CRC patients	Patients with CRC	Metformin user <i>vs</i> nonuser	HR: 0.81 (0.60-1.08)
Skinner <i>et al</i> [100], 2013	Retrospective cohort. 482 locally rectal cancer patients	Locally advanced rectal adenocarcinoma treated with chemoradiation and surgery	Metformin user <i>vs</i> nonuser	pCR: OR: 16.8 (1.6-181.1). OS at 5 and 10 years (metformin <i>vs</i> non): 81% and 79% <i>vs</i> 56% and 39% ($P = 0.022$)
Miranda <i>et al</i> [101], 2016	Phase 2 Clinical trial. 50 refractory CRC patients	Refractory CRC patients	Metformin 850 mg twice a day+ 5-FU 425 mg/m ² weekly	PFS: 1.8 mo. OS: 7.9 mo. Obese <i>vs</i> lean: 12.4 <i>vs</i> 5.8 mo
Bragagnoli <i>et al</i> [102], 2021	Phase 2 Clinical trial, 41 refractory CRC patients	Refractory CRC patients	Metformin 2500 mg a day+ Irinotecan 125 mg/m ² D1, D8, every 21 d	PFS: 2.4 mo, CI 95%, 2.0-4.5 mo. OS: 8.4 mo, CI 95%, 5.9-10.8 mo
El-Fatraty <i>et al</i> [103], 2018	Clinical Trial, 40 Stage III CRC patients	Stage III CRC patients	FOLFOX 4 12 cycles + metformin 500 mg 3 times a day	Neuropathy grade 2-3 (metformin <i>vs</i> non): 60% <i>vs</i> 95% ($P = 0.009$)
Gastric cancer				
Lee <i>et al</i> [118], 2016	Retrospective Cohort, single center in Korea. 1974 GC resected patients: - 132 DM2 with metformin; -192 DM2 without metformin; -1648 non-diabetic	GC patients who underwent curative gastrectomy	Metformin user <i>vs</i> nonuser	OS-HR: 0.58 (0.37-0.93). RFS-HR: 0.63 (0.41-0.98)
Lacroix <i>et al</i> [120], 2018	Retrospective Cohort. 371 Patients	Stage I to III GC patients	Metformin user <i>vs</i> nonuser	OS-HR: 0.73 (0.52-1.01); cancer specific mortality-HR: 0.86 (0.56-1.33)
Baglia <i>et al</i> [121], 2019	Prospective cohort study in Shanghai. 543 GC patients	Breast, CRC, lung and GC patients	Metformin user <i>vs</i> nonuser	OS-HR: 1.11 (0.81-1.53). Disease-specific survival-HR: 1.03 (0.73-1.43)
Seo <i>et al</i> [119], 2019	Retrospective cohort study. 2187 GC resected patients: - 103 DM2 with metformin; -139 DM2 without metformin; -1945 non-diabetic	GC patients who underwent curative gastrectomy	Metformin user <i>vs</i> nonuser	HR: 0.45 (0.30-0.66)
PDAC				
Sadeghi <i>et al</i> [128], 2012	Retrospective cohort. $n = 302$	DM2 patients. All stages. United States single center	Metformin user <i>vs</i> nonuser	HR: 0.64 (0.48-0.86)
Chaiteerakij <i>et al</i> [129], 2016	Retrospective cohort. $n = 980$	DM2 patients. All stages. United States single center	Metformin user <i>vs</i> nonuser	HR: 0.93 (0.81-1.07)
Lee <i>et al</i> [133], 2016	Retrospective cohort. $n = 237$	DM2 patients. All stages. Korean single center	Use of metformin ≥ 1 -mo post-diagnosis <i>vs</i> nonuser	HR: 0.61 (0.46-0.81)
Ambe <i>et al</i> [130], 2016	Prospective cohort study $n = 44$	DM2 patients. Resected PDAC, stage I-II. United States single center	Metformin user <i>vs</i> nonuser	HR: 0.54 (0.16-1.86)
Cerullo <i>et al</i> [131], 2016	Retrospective cohort. $n = 3393$	Resected PDAC United States population based	Metformin use after surgery <i>vs</i> nonuser	HR: 0.79 (0.67-0.93)
Jang <i>et al</i> [132], 2017	Prospective cohort. $n = 764$	DM2, OHA user. Resected Korean population based	Metformin user <i>vs</i> nonuser	HR: 0.73 (0.61-0.87)
Hwang <i>et al</i> [135], 2013	Retrospective cohort. $n = 516$	DM2 patients. Locally advanced and metastatic. United Kingdom population based	Use of metformin peridiagnosis <i>vs</i> nonuser	HR: 1.11 (0.89-1.38)
Choi <i>et al</i> [134], 2016	Retrospective cohort. $n = 183$	DM2 patients. Locally advanced and metastatic. Korean single center	Metformin user <i>vs</i> nonuser	HR: 0.69 (0.49-0.97)
Kordes <i>et al</i> [137], 2015	RCT, $n = 121$	Locally advanced and metastatic. Multicentric. Netherlands	Gemcitabine-everolimus (1000 mg/m ² D1, 8, 15-every 28 d-1.000 mg/d) +/- metformin (2000 mg/d)	HR: 1.05 (0.72-1.55)
Reni <i>et al</i> [138], 2016	RCT. $n = 60$	Metastatic. Single center. Italian	PEXG (cisplatin-epirubicin-capecitabine-gemcitabine: 30 mg/m ²	HR: 1.56 (0.87-2.80)

			D1,14- 30 mg/m ² D1,14-2500 mg/m ² D1-28 - 800 mg/m ² D1-14) +/- metformin 2000 mg/d	
Zhou <i>et al</i> [136], 2017	Meta-analysis 12 cohort studies and 2 RCT. <i>n</i> = 94778	Studies that investigated metformin exposition. All stages PDAC	Metformin user <i>vs</i> nonuser	HR: 0.77 (0.68-0.87)
Li <i>et al</i> [139], 2017	Meta-analysis. 9 cohort study and 2 RCT. <i>n</i> = 8089	Studies that investigated metformin exposition. All stages PDAC	Metformin user <i>vs</i> nonuser	HR: 0.86 (0.76-0.97)
Wan <i>et al</i> [140], 2018	Meta-analysis 15 cohort studies and 2 RCT, <i>n</i> = 36791	Studies that investigated metformin exposition. All stages PDAC	Metformin user <i>vs</i> nonuser	HR: 0.88 (0.80-0.97). Asians only HR: 0.74 (0.58-0.94); Stage I-II HR: 0.76 (0.68- 0.86); Stage III-IV HR: 1.08 (0.82-1.43)
Braghiroli <i>et al</i> [141], 2015	Single-arm phase II. <i>n</i> = 20	Locally advanced or metastatic. 2 nd line treatment. Single center. Brazilian	Paclitaxel (80 mg/m ² D1, 8, 15 every 28 d) + metformin 1750 mg/d	DCR at 8 wk 31, 6%
Pancreatic neuroendocrine tumor				
Pusceddu <i>et al</i> [153], 2018	Retrospective cohort. <i>n</i> = 445	Locally advanced or metastatic. Multicentric. Italian	No DM2 <i>vs</i> DM2. Metformin user <i>vs</i> nonuser	HR: 0.45 (0.32-0.62). HR: 0.49 (0.34-0.69)
Hepatocellular carcinoma				
Chen <i>et al</i> [163], 2011	Retrospective cohort. <i>n</i> = 53	DM2. Early-stage HCC. RFA treated. Single center. Taiwanese	Metformin user <i>vs</i> nonuser	HR: 0.24 (0.07-0.90)
Ma <i>et al</i> [164], 2016	Meta-analysis. 11 cohort studies. <i>n</i> = 3452	Studies that investigated metformin exposition. HCC patients	Metformin user <i>vs</i> nonuser	HR: 0.59 (0.42-0.83)
Intrahepatic cholangiocarcinoma				
Yang <i>et al</i> [169], 2016	Retrospective cohort. <i>n</i> = 250	DM2. Newly diagnosed ICC. United States single center	Metformin user <i>vs</i> nonuser	HR: 0.80 (0.60-1.20)

PDAC: Pancreatic ductal adenocarcinoma; ORC: Origin recognition complex; HR: Hazard ratio; PCR: Polymerase chain reaction; OS: Overall survival; PFS: Progression-free survival; RFS: Regarding refeeding syndrome; OHA: Oxidized hyaluronate; RCT: Randomized clinical trial; PEXG: Pseudoexfoliative glaucoma; DCR: Dacryocystorhinostomy; HCC: Hepatocellular carcinoma; RAF: Rapidly accelerated fibrosarcoma; ICC: Intrahepatic cholangiocarcinoma; CRC: Colorectal cancer; DM2: Diabetes mellitus type 2; GC: Gastric cancer.

et al[106] showed *in vitro* that metformin and cisplatin in combination decreased the effects of cisplatin alone[106].

In recent years, several observational studies have shown that metformin reduces the risk of GC[107-112]. The study of Tseng *et al*[107] demonstrated that GC risk was reduced using metformin, especially when the cumulative duration was more than 2 years[107]. In addition, metformin reduced the risk of GC, while opposite results were observed with sulfonylureas[108].

On the other hand, a study conducted in United Kingdom did not show a difference in GC incidence in patients receiving metformin compared to sulfonylureas[113]. Other reports also could not find any reduction in GC risk associated with metformin use[83,114,115]. Despite that, a meta-analysis showed a 21% reduction in the risk of GC with the use of metformin, in Asians the benefit was more prominent than in Westerners[116]. Another meta-analysis of cohort studies that included 591077 patients found a significantly lower incidence of GC with metformin therapy than other types of therapy (HR: 0.763; 95%CI: 0.642-0.905)[117].

Two retrospective studies conducted by Lee *et al*[118] and Seo *et al*[119] concluded that metformin reduced GC recurrence in patients undergoing gastrectomy[118,119]. Lacroix *et al*[120] showed that metformin improved OS but not cancer specific survival, in contrast, Baglia *et al*[121] observed that metformin use did not impact patient's survival[120,121].

More studies are needed to confirm the effect of metformin in GC treatment and chemoprevention. Unfortunately, there are few clinical trials that are ongoing to analyze this question. An interesting phase 2 randomized trial (NCT04114136) are ongoing to evaluate the synergistic effect of metformin, rosiglitazone and anti-PD-1 on

the treatment of refractory solid tumors including GC. Metformin could reduce tumor oxygen consumption creating a less hypoxic T cell environment leading to restore its anti-tumor cell function. The trial NCT04033107 analyze the combination of metformin and vitamin C in DS tumors including GC.

Metformin and pancreatic cancer

Pancreatic cancer is the fourth leading cause of cancer death in the United States and its prognosis remains dismal, encouraging research to discover innovative agents active in its treatment is an urgent unmet need[122]. Pancreatic ductal adenocarcinoma (PDAC) is its most common histologic type. An association between metformin use and decreased PDAC incidence in patients with DM2 was first recognized by two large clinical studies. In a large general practice retrospective cohort, Currie *et al*[123] reported risk reduction in metformin users related to sulfonylurea users (HR: 0.20; 95%CI: 0.11-0.36) and to insulin-based-treatment users (HR: 0.22; 95%CI: 0.12-0.38). Likewise, in a hospital-based case-control study, Li *et al*[124] encountered risk reduction in metformin users compared to those who did not use metformin (OR: 0.38; 95%CI: 0.21-0.67). Several meta-analyses have strongly reinforced PDAC risk reduction with metformin use in patients with DM2[18,125-127]. However, this effect should prospectively be confirmed in large prospective clinical trials.

Regarding survival, in a retrospective study, Sadeghi *et al*[128] reported a 36% lower risk of death (HR: 0.64; 95%CI: 0.48-0.86), OS benefit of 4 mo (15.2 mo *vs* 11.1 mo) and approximately 2-fold increase in 2-year survival rate (30.1% *vs* 15.4%) in patients who took metformin compared to those inpatients who did not take metformin. Interestingly, longer survival was only observed in non-metastatic disease, when stratified by disease stage[128]. Further evidence also encountered survival improvement in the subgroups of resected or locally advanced but not in patients with metastatic disease[129]. Specifically, among resected patients with PDAC, metformin use seemed to improve OS after 18 mo[130-132]. Related to locally advanced or metastatic disease, further evidence was contradictory on survival gains in patients with PDAC exposed to metformin with benefit being reported only in an Asian cohort[133-135]. A large meta-analysis analyzed data from 12 retrospective cohorts demonstrating OS improvement in metformin users at various stages (HR: 0.77; 95%CI: 0.68-0.87)[136].

Stimulated by this retrospective evidence, two European groups explored, in randomized clinical trials (RCTs), the association of metformin with gemcitabine-based chemotherapy as first-line treatment of advanced PDAC with negative results on OS improvement[137,138]. Recently, a meta-analysis, with inclusion of two RCTs, re-analyzed the improvement in OS and confirmed benefit in the whole population of diabetic patients with PDAC (HR: 0.86; 95%CI: 0.76-0.97)[139]. Analysis of subgroups in this study demonstrated improved survival in patients with resected or locally advanced tumors but not in the metastatic group. Similar results were observed in another group with a benefit in OS at various stages, which was more evident in the subgroups of less advanced stages and Asian patients[140]. Considering second-line treatment, a single arm prospective study did not reach survival gain of metformin associated to paclitaxel[141]. Results of ongoing clinical trials recently completed are expected with substantial interest. NCT01666730 explores overall survival improvement of metformin associated with modified FOLFOX6 in metastatic patients, NCT02005419 evaluates DFS at 1 year with the combination of metformin and gemcitabine in resected subjects and NCT02048384 analyses safety of metformin with or without rapamycin after disease stabilization on first line chemotherapy in metastatic individuals.

This clinical evidence is associated with the pre-clinical data that pancreatic cancer cells are sensitive to inhibition of oxidative phosphorylation, decreases in insulin-IGF signaling and inhibition of the mTOR pathway through AMPK activation, which are some of the major antineoplastic effects of metformin[39,142-145]. Identifying predictive or prognostic factors of response to metformin should be of relevance to select patients most likely to benefit from the effects of metformin[39]. Recent advances in molecular characterization might distinguish different biology and response to therapy in patients with morphologically similar PDAC and may be incorporated into clinical trials[146-148]. Moreover, the recently experienced challenge of standard of care in advanced pancreatic cancer treatment with polychemotherapy also brings new perspectives, as patients experience longer survival with the need to combine other active agents[149,150]. Future trials would include disease stage, identification of biomarkers and concentrations of metformin in neoplastic tissue to powerfully evaluate the benefit of metformin in the treatment of PDAC.

Another pancreatic neoplasm with rising incidence is pancreatic neuroendocrine tumors (panNETs)[151]. Few studies have evaluated the clinical benefit of metformin in the treatment of panNETs[152]. Pusceddu *et al*[153], in a multicentric retrospective cohort of patients receiving everolimus with or without somatostatin analogues, reported increased PFS in diabetic patients exposed to metformin compared to diabetic patients not exposed to metformin or non-diabetic patients [44.2 vs 20.8 mo (HR: 0.49; 95%CI: 0.34-0.69) or 15.1 mo (HR: 0.45; 95%CI: 0.32-0.62), respectively][153]. This result correlates with *in vitro* evidence that metformin decreases proliferation in human panNET cell lines[154,155]. A recent study demonstrated that the combination of metformin and everolimus strongly inhibited human panNET cell proliferation through mTOR suppression, compared to each agent used alone[156]. Results of the ongoing NCT02294006 prospective trial are expected to better evaluate the effects of this experimental treatment on PFS at 12 mo.

Metformin and hepatocellular carcinoma

The incidence of hepatocellular carcinoma (HCC) has strongly increased in last two decades, as well as the prevalence of its metabolic risk factors[156,157]. Hassan *et al*[158] and Donadon *et al*[159], in hospital-based case-control studies, first observed the strong association of metformin use and reduced risk of HCC in subjects with DM2 (HR: 0.15; 95%CI: 0.04-0.50) (HR: 0.30; 95%CI: 0.20-0.60)[156,158,159]. This protective effect was validated by accumulated evidence of observational studies including more than 0.5 million subjects (OR: 0.52; 95%CI: 0.40-0.68), being more evident in case-control than in cohort studies and without significance in the *post hoc* analysis of RCTs[160-162]. These data suggest an association between metformin use and reduced HCC incidence that needs to be confirmed in prospective clinical trials.

Improvement in HCC survival was first reported by Chen *et al*[163] in an early-stage cohort of patients treated with radiofrequency ablation with longer OS in metformin users compared to non-users (HR: 0.24; 95%CI: 0.07-0.90)[163]. A meta-analysis of 11 cohort studies was in accordance with better prognosis related to metformin use in patients with HCC related to their counterparts (HR: 0.59; 95%CI: 0.42-0.83)[164].

Although the antineoplastic effects of metformin in liver cancer are not completely understood, pre-clinical evidence observed inhibition of proliferation and induction of cell cycle arrest and apoptosis in HCC cells through AMPK activation[165,166]. Future prospective trials should explore the potential benefit of metformin in prevention and treatment of HCC.

Metformin and intrahepatic cholangiocarcinoma and gallbladder cancer

Intrahepatic cholangiocarcinoma (ICC) is the second most common hepatic cancer, and its incidence has markedly increased in the last decades[167]. Chaiteerakij *et al*[168], in a clinic-hospital-based retrospective cohort, reported 60% reduced risk of ICC in patients with DM2 who used metformin related to non-users (OR: 0.4; 95%CI: 0.2-0.9)[168]. The same group, however, did not encounter better prognosis in patients with DM2 with ICC taking metformin (HR: 0.8; 95%CI: 0.6-1.2)[169]. Although gallbladder cancer (GBC) is the most common biliary tract cancer[170], no clinical data and scarce basic evidence have explored the antineoplastic effects of metformin and its potential mechanisms of action in GBC.

Regarding comprehension of the possible mechanistic effects of metformin on ICC and GBC there are some *in vitro* and *in vivo* evidence. Overall, the studies observed the induction of apoptosis and cell cycle arrest mediated by activation of the AMPK-mTOR axis[171-173]. The association of metformin in combination with gemcitabine and cisplatin (the standard of care for advanced ICC) enhanced the antiproliferative effects of treatment in a cell model through their effects on AMPK, cyclin D1 and caspase-3[174]. Furthermore, Liu *et al*[175] first observed the decreased survival of GBC cells *via* inhibition of phosphorylated Akt (p-Akt) and Bcl-2 signaling[175]. Likewise, metformin inhibited GBC cell proliferation *via* downregulation of HIF-1 α and VEGF and promoted cell cycle arrest by reduction of cyclin D1 expression in different animal experiments[176,177]. The association of metformin with cisplatin also promoted reduced expression of p-Akt and cyclin D1 downregulation, resulting in a synergistic antiproliferative effect in GBC cells[178].

These pre-clinical and preliminary clinical evidence highlights the need for metformin to be more deeply explored in clinical studies of ICC and GBC prevention and treatment. Considering the rationale that metformin may be active in the prevention and treatment of ICC and limited clinical data, exploratory studies should address this issue for a better understanding of its benefit in these clinical settings.

PERSPECTIVES

DS tumors are often associated to high morbidity and mortality and their incidence has increased over recent decades[179]. Recognition of its main risk factors and conditions of worse prognosis as well as development of strategies for prevention and treatment urges. In this context, projection of a worldwide burden of cancer attributable to diabetes and excess weight for the near future is an alarming public health concern[180]. Association of several cancers, including many DS tumors to diabetes and obesity have already been recognized (IARC, WCRF). Further strategies of prevention and treatment urge to be known. The large amount of evidence presented herein supports the idea of an important effect of metformin in decreasing risk and improving prognosis of several DS tumors.

Although most clinical studies presented here are retrospective that are often limited by immortal time and selection bias, recent discoveries of pre-clinical research on antineoplastic effects of metformin establish biological plausibility for the clinical data and reinforce the interest on its effects in carcinogenesis and cancer progression. These preclinical and clinical evidence supports running of adequately powered trials to investigate clinical use of metformin on DS tumors treatment. This should consider diabetic status, predictive biomarkers, disease stage and treatment setting. Concerning chemoprevention, safety, low cost, and widespread access are key to its feasibility. Therefore, repurposing metformin for DS cancer treatment is a scientific field of remarkable interest as it focuses on a global public health problem.

Currently, clinical research is considered a job with its inherent needed professional skills[181]. Taking in consideration that the low metformin cost does not impact the expensive process of drug repurposing, the development of this potential anti-cancer drug has been hampered. Moreover, the current stage of metformin clinical development needs testing in large, randomized, genome-guided, multicenter trials. These aspects explain, at least in part, the shortage of current studies on metformin in cancer prevention and treatment despite the large number of pre-clinical and clinical evidence indicating its potential benefit. We hope that this comprehensive review integrating the potential mechanisms, pre-clinical and clinical studies of metformin as anticancer agent alert the DS cancer community for the need of studying metformin effects in more specific clinical scenarios.

CONCLUSION

The remarkable intracellular pathway change caused by oncogenesis and the potential mechanisms of the antitumoral action of metformin have been supported. They have revealed novel target molecules and newly discovered treatment possibilities. In connection with epidemiological, pre-clinical, and clinical research, data support that metformin benefits some patients with DS tumors, requiring strict clinical trials to identify those who might obtain advantage from metformin combinations. Given that the survival outcomes are affected by a multitude of factors, such as cancer type, differentiation, staging and treatment, for adequately repurposing the use of metformin in DS cancers it is essential to take into consideration patient characteristics that may serve as predictive biomarkers of metformin antitumoral effects, such as insulin resistance, diabetes, body composition, and chronic diseases related to inflammation, as well as the specific tumor driven oncogenic pathway, which may interfere with the direct and indirect antitumoral effects of metformin.

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