Chapter

Repurposing of Metformin as a Multifaceted and Multitasking Preventative and Treatment for Cancer

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Abstract

Metformin is a cornerstone treatment of diabetes mellitus. Since 2005 when it has been first reported to reduce the risk of cancer in diabetics, a large number of preclinical and clinical studies have implicated its potential role as a preventative and adjunct therapy for a broad range of cancers. Whereas preclinical studies demonstrate its actions on a multitude of molecular pathways involving nearly all aspects of cancer development including metabolism, angiogenesis, apoptosis, autophagy, immunity, epigenetics, inflammation and crosstalk with the microbiome, other studies demonstrate its synergism with a range of anticancer modalities including chemotherapy, radiotherapy, immunotherapy, and targeted therapies. Furthermore, an increasing number of clinical studies not only confirm its preventative properties against cancers but have extended its potential for a possible adjunctive role in the neoadjuvant, adjuvant, maintenance and salvage therapies of cancer. This article intends to summarize the basic science that allows us to understand the complex multiple mechanisms of action of this remarkable multitasking molecule as well as review the recent meta-analyses that have summarized the clinical studies assessing the therapeutic efficacy of metformin for various cancers.

Keywords: metformin, diabetes, repurposing, cancer therapy

1. Introduction

Metformin is derived from the French lilac (also known as goat's rue or *Gallega Officinalis*), a medieval European medicinal herb that was first described as a diabetes treatment in a mid-17th century English treatise called *Culperper's Complete Herbal*, but it was not until 1957 that the French physician Jean Sterne formally patented metformin as a drug treatment for diabetes. The efficacy of metformin for type 2 diabetes mellitus (T2DM) has since been established and it was approved by the US FDA in 1995 as a treatment for T2DM. Meanwhile by the late 1980's, studies on the effects of metformin on insulin receptor binding on tumor cells led researchers to conceive that metformin's effect may potentially be applied for cancer management [1]. Separately, it has long been suspected that T2DM may be a risk for cancer with its cancer promoting effects believed due to hyperinsulinemia in T2DM, since insulin was believed to exert a mitogenic effect [2], thus it was simply logical to investigate the potential

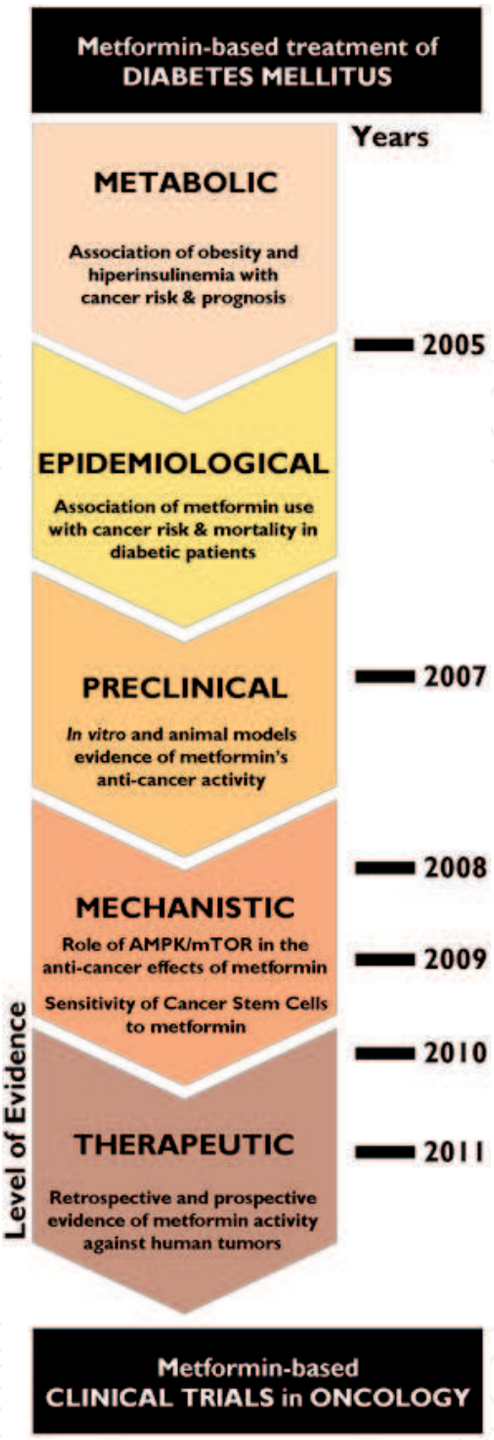

Figure 1.
Research development of metformin as anticancer agent: Since early epidemiologic reports suggesting metformin use in type 2 diabetes was associated with reduced cancer incidence, research evidence that metformin may be preventive and/or therapeutic for human cancers has expanded, with most of the molecular and clinical breakthroughs in metformin and cancer have taken place during the past decades, and hundreds of clinical trials are currently exploring metformin's potential in cancer. Source: [5], Licensed under CC BY 3.0.

benefits of an insulin lowering and hence counter-mitogenic anti-diabetic agent for its possible anti-cancer effects. By the early 2000's, studies have already established the potential benefits of metformin on hyperinsulinemia, obesity, hyperlipemia, hypertension, fibrinolysis, and endothelial dysfunction, with the expansion of the drug's potential applicability beyond T2DM to address weight gain, acanthosis nigricans, infertility and polycystic ovary syndrome [3]. In 2005, a landmark retrospective case control study by Evans et al. demonstrated that metformin exposure in T2DM was associated with the reduced risk of cancers [4] and further epidemiological studies

also corroborated that diabetics treated with metformin have a lowered incidence of cancer than those treated with other agents, leading to increasing calls for the use of metformin to reduce the risk of cancer. In the past decade, metformin has seen over 50 million prescriptions per year in the US alone and there has been a concurrent explosion of interest in metformin's anticancer effects with dozens of systematic reviews and meta-analyses performed and published on hundreds of cancer studies involving hundreds of thousands of patients and with hundreds of clinical trials on metformin and cancer currently actively recruiting. The development and expansion of research into metformin's anticancer activities in the past two decades from the bench to the clinic is illustrated below in **Figure 1**.

Given that this review is intended as a summary of current clinical evidence for the potential uses of metformin in the prevention and treatment of cancer, we will provide only a succinct synthesis of the thousands of preclinical studies on the biological mechanisms and molecular pathways that has been performed in the past two decades and focus our attention mostly on recent clinical evidence of metformin's efficacy as demonstrated by clinical studies.

2. Pleiotropic effects of metformin against cancer

The early days of laboratory research on metformin's anti-cancer mechanisms focused mainly on its metabolic effects on cell proliferation, which naturally follows from the initial use of metformin as a treatment for T2DM as a metabolic disorder. Eventually, it became gradually apparent that unlike modern day targeted therapies, metformin's anti-neoplastic bioactivity is broad ranged and pleiotropic, encompassing not only its established metabolic effects, but also involving antiangiogenic, anti-inflammatory, epigenetic, apoptotic and autophagic, and immunologic actions as well as effects on the microbiome and on cancer stem cells (CSCs) that all synergistically contribute to overall cancer prevention and control. Furthermore, within each category of its bioactivity, it further exerts multiple molecular actions, and it has thus become increasingly apparent that metformin could be properly conceived of as a multi-faceted multi-tasking molecule with direct and indirect actions against cancer. In summary, the anticancer effects of metformin is based on 1) its main action on cellular metabolism via the maintenance of plasma glucose and insulin levels, 2) targeted action against cancer cells with pleiotropic inhibitory effects on multiple pathways involved in cancer cell survival and metastasis, and 3) indirect anti-angiogenic anti-inflammatory as well as immunomodulatory effects and also its actions on the microbiome and CSCs. The complex pleiotropic nature of metformin effects on cancer is illustrated in **Figure 2**.

2.1 Metformin metabolic effects

To understand the metabolic impact of metformin on cancer, we must first recognize the intimate relationship between glucose energy metabolism and cellular proliferation as well as a unique propensity of cancer cells to utilize glucose anaerobically even in the presence of oxygen in contrast to non-cancer cells which utilize oxidative phosphorylation to generate energy. This phenomenon was first noted by Otto Warburg almost a hundred years ago, and subsequently termed the "Warburg effect" [7]. This altered energy metabolism of cancer cells may underline their proliferation, invasiveness, and chemoresistance and this altered metabolic pattern in cancer is regulated by oncogenic and tumor suppressor signals such as hypoxia inducible factor 1 (HIF-1), myelocytomatosis oncogene cellular homolog

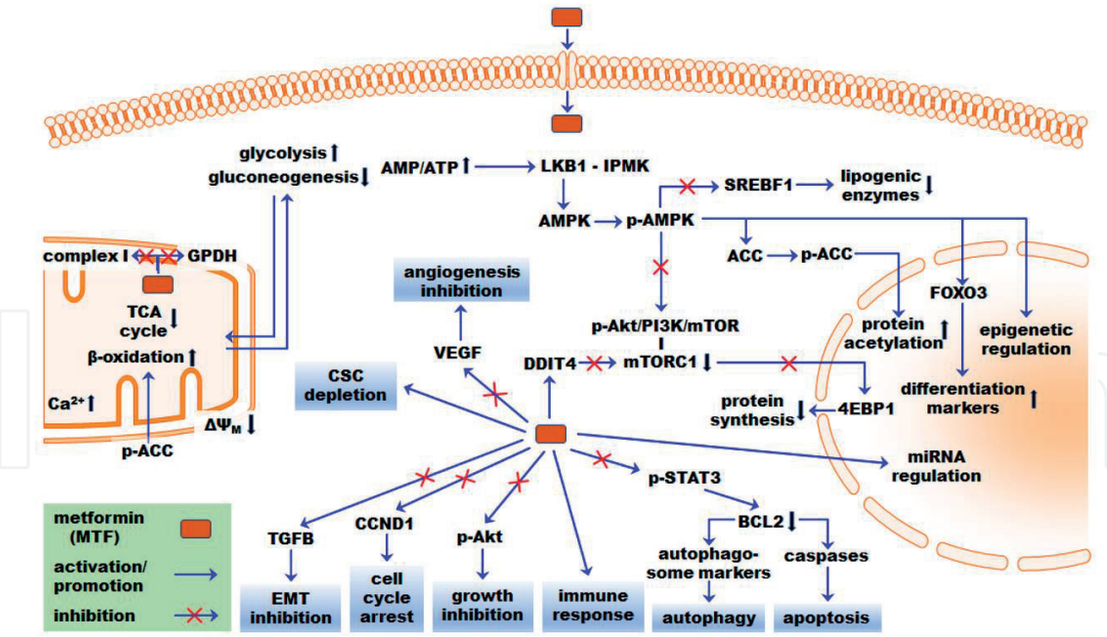

Figure 2.
Representation of some of the pleiotropic direct and indirect anticancer effects of metformin as illustrated by molecular and cellular pathways. Metformin effects key energy and metabolic processes such as the mitochondrial respiration (complex I), TCA cycle, fatty acid β-oxidation, gluconeogenesis, and glycolysis. Metformin affects the cell cycle, cell growth, immune response, autophagy, and apoptosis, angiogenesis and cancer stem cells. Abbreviations: 4EBP1, 4E-binding protein 1; ACC, acetyl-CoA carboxylase; AKT, AKT serine/threonine kinase 1; AMPK, AMP-activated protein kinase; BCL2, apoptosis regulator, BCL2; CCND1, cyclin D1; CSC, cancer stem cell; DDIT4, DNA damage inducible transcript 4; EMT, epithelial-to-mesenchymal transition; FOXO3, forkhead box O3; GPDH, glycerol-3-phosphate dehydrogenase; IPMK, inositol polyphosphate multikinase; LKB1, liver kinase B1; miRNA, micro RNA; mTORC1, target of rapamycin complex 1; SREBF1, sterol regulatory element binding transcription factor 1; STAT3, signal transducer and activator of transcription 3; TCA, tricarboxylic acid; TGFB1, transforming growth factor beta 1; VEGF, vascular endothelial growth factor. Phosphorylated molecules are indicated by a prefix p. source: [6], Licensed under CC BY 4.0.

(Myc), p53, and the phosphoinositide 3 kinase (PI3K)/AKT8 virus oncogene cellular homolog (Akt)/mammalian target of rapamycin (mTOR) pathways.

Metformin's main pharmacologic action is the reducing elevated plasma glucose is largely due to the improvement in hepatic insulin resistance leading to a reduction in hepatic glucose output from gluconeogenesis, increases glucose uptake in muscle, decreased absorption of sugar from the intestines, and improved insulin sensitivity, mainly via activation of a cellular energy sensor known as AMP-activated protein kinase (AMPK). The major downstream target of AMPK is mTOR, which is very important in cellular growth processes and cancer dynamics, and mTOR is inhibited by AMPK [8]. Since glucose metabolism is at the center of the metabolic derangement that is a hallmark of cancer cells, and metformin chiefly targets glucose metabolism, it follows that the altered metabolic pathway may be a target by metformin for cancer prevention or therapy.

It is through its main effects above on metabolism and cellular energetics that metformin can attenuate cancer cell proliferation (See **Figure 3**). Furthermore, these metabolic effects in turn impact the immune system, epigenetics, inflammation, cellular apoptotic and autophagic pathways as well as the microbiome and CSCs which all play a role in cancer development.

2.2 Metformin immuno-modulatory effects

The immune system participates broadly in the prevention and control of cancer and interacts with biological pathways of metabolism and inflammation,

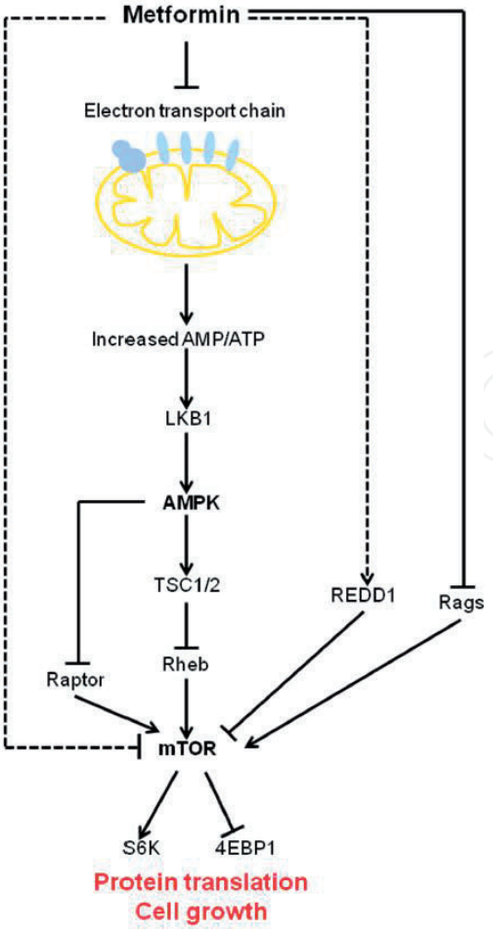

Figure 3.
The effect of metformin in suppressing cancer cell growth via metabolic pathways. Metformin inhibits complex I of the electron transport chain, which leads to increased AMP/ATP ratio and activation of AMPK by LKB1. Activated AMPK subsequently inhibits mTOR and its downstream targets by the following two pathways: 1. AMPK stabilizes TSC1/2, which inhibits Rheb, an activator of mTOR; 2. AMPK inhibits mTOR binding protein raptor. Metformin directly inhibits mTOR by up-regulating REDD1 and suppressing rags. AMPK, AMP-activated protein kinase; Rheb, Ras homolog enriched in brain; LKB1, liver kinase B1; REDD1, regulated in development and DNA damage response 1; TSC, tuberous sclerosis complex; rags, rag GTPases; mTOR, mammalian target of rapamycin; 4EBP1, eukaryotic initiation factor 4E binding protein 1; S6K, S6 kinase. Source: [9], Licensed under CC BY 3.0.

and metformin again acts in a multifaceted fashion to bolster immunity against cancer with effects on almost every aspect of the immune system, especially with reference to cancer immunty (**Figure 4**). One of metformin's actions is the enhancement of CD8+ T lymphocytes and rescues them from exhaustion. CD8+ T cells which is one of the key components in cellular immunity against tumors, as these cells can expand and transform into effector cytotoxic T lymphocytes (CTL) which targets cancer. This phenomenon of the rescue of exhausted CD8+ T lymphocytes has been confirmed *in vitro* in leukemia, melanoma, renal cell carcinoma, non–small-cell lung carcinoma (NSCLC), gastrointestinal carcinoma, and breast cancer. Also, metformin-induced activation of AMPK as one of its main metabolic actions mentioned above promotes immune check-point programmed death ligand 1 (PD-L1) degradation, which allows CTL-mediated tumor cell death [11]. Additionally, metformin can also enhance local as well as systemic cytokine responses to tumors [12]. Furthermore, metformin also has indirect effects on the immune system via its influence on the microbiome and its anti-inflammatory effects, which has been reviewed exhaustively and is briefly summarized below.

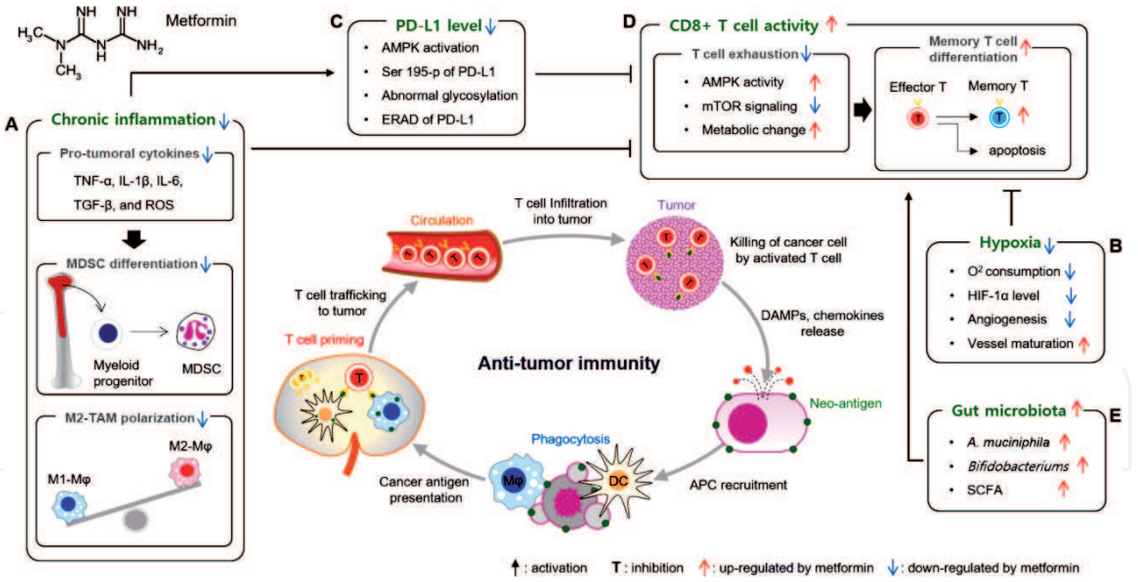

Figure 4.
Metformin effects related to anticancer immunity. Metformin indirectly increases T-cell activity by negatively regulating (a) chronic inflammation, (B) hypoxia, and (C) PD-L1 levels that inhibit T-cell activity. Metformin directly relieves T-cell exhaustion by means of metabolic reprogramming of TIL and promotes memory T-cell differentiation (D). Metformin shifts the profile of gut microbiota more favorably to T-cell immunity (TAM) tumor-associated macrophages (E); (Mφ) macrophages; (MDSC) myeloid-derived suppressor cells; (T) T-cell; (DAMPs) damage-associated molecular patterns; (APC) antigen presenting; (SCFA) short-chain fatty acid. Source: [10], CC BY-NC 3.0.

2.3 Metformin effects on the microbiome

Whereas science has become increasingly aware of the central role the gut microbiome plays in health and diseases including cancer, particularly via its effects on the immune system [13], metformin's beneficial role on host metabolism has also been found to be in part related to the microflora in the gut. The microbiome modulates our immune system and inflammatory response and both of these are key factors in determining cancer development and are associated with inflammatory immune response [14] highlights the crosstalk between metformin effects on metabolism, immunity, inflammation and the microbiome, which in turn can modulate cancer biodyamanics, and part of the mechanisms involved in this complex interplay is illustrated in **Figure 5** below.

2.4 Metformin anti-inflammatory effects

Inflammation effects on cancer promotion is well known. In 1863, Rudolf Virchow first proposed the role of inflammation in cancer based on the observation of leukocytes in cancerous tissue. Subsequently, accumulated evidence has identified inflammation both as a cause and result of malignancy [16], with numerous studies in past decades implicating chronic inflammation in the promotion of malignancy [17] (**Figure 6**). Not surprisingly then, given the T2DM's known association with chronic low-grade subclinical inflammation which is part and parcel of its the insulin resistance that is its hallmark [19], and metformin's effects on the immune and metabolic systems, that metformin must also modulate the inflammatory response. This connection has been well demonstrated by animal experiments where metformin treated rodents reveal dampened pro-inflammatory pathways nuclear factor k B (NF-k) and Jun N-terminal kinase (JNK) and increased anti-inflammatory cytokine IL-10 [20].

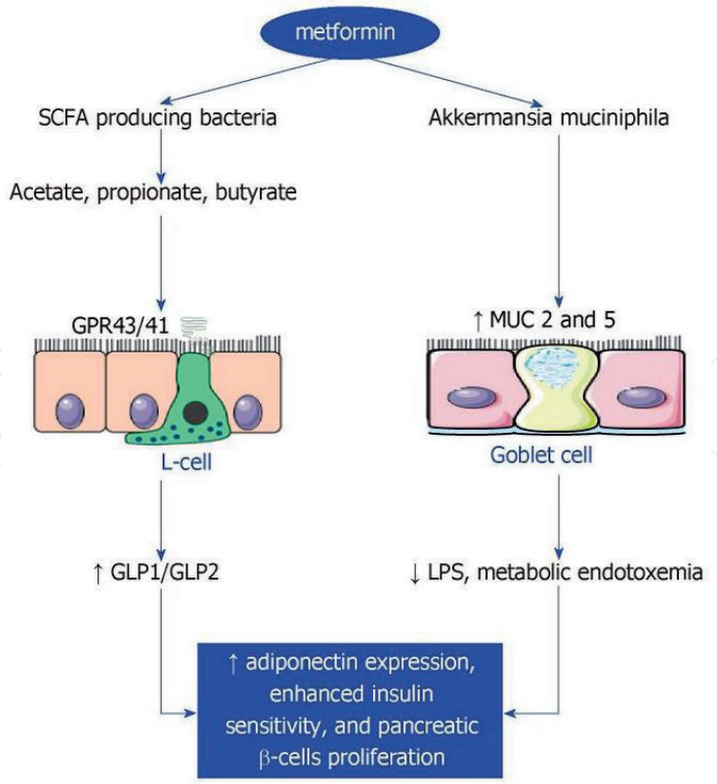

Figure 5.
Crosstalk between metformin action and gut microbiota. GLP1: Glucagon-like peptide-1; GLP2: Glucagon-like peptide-2; LPS: Lipopolysaccharide; SCFA: Short-chain fatty acid. Source: [15], Licensed under CC BY-NC 4.0.

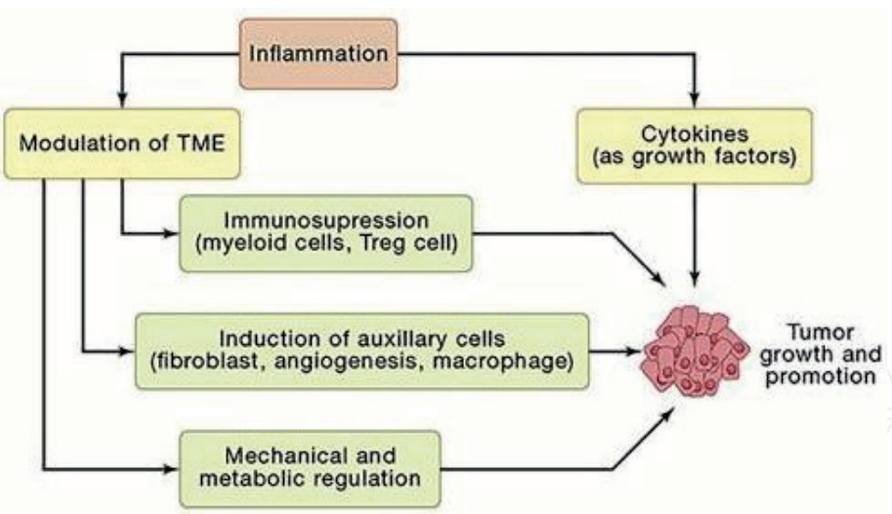

Figure 6.
Inflammatory cytokines released by immune cells within the tumor microenvironment has a direct effect on pre-malignant and cancer cells by increasing their proliferation and resistance to cell death and stresses thus directly promoting tumor growth and progression. Additionally, inflammatory signals can suppress anti-tumor immunity via action of regulatory T-cells, myeloid cells and enhance other cancer promoting cells (such as fibroblasts, myeloid cells and endothelium of new blood vessels); altogether, these inflammation driven changes also significantly contribute to tumor growths and progression. TME: Tumor microenvironment, Treg: Regulatory T cells. Source: [18], Licensed under CC BY 3.0.

2.5 Metformin epigenetic effects

Epigenetics is the genomic mechanism that reversibly modulates gene expression independent of DNA sequences. Epigenetic processes which allow for the gene modulatory effect involve DNA methylation, histone modification,

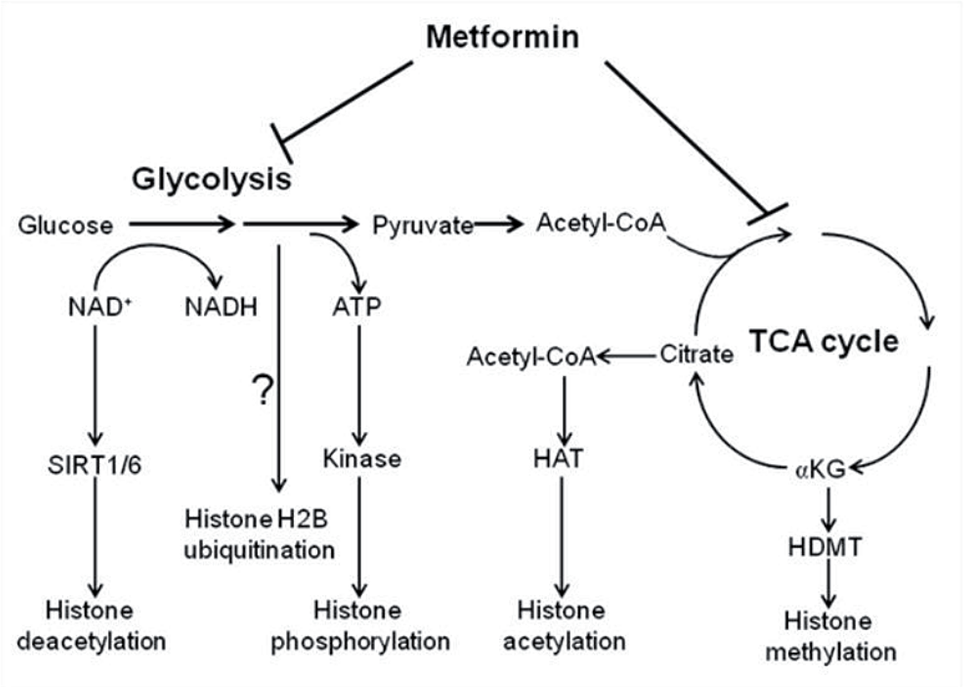

Figure 7.
Schematic of histone modifications via metabolic effects of metformin. Glycolysis determines the NAD⁺/NADH ratio, which affects the activity of histone deacetylases to reduce histone acetylation. Source: [9], Licensed under CC BY 3.0.

the readout of these modifications, chromatin remodeling and the effects of noncoding RNA all of which affects cellular activities such as growth and differentiation. Thus, epigenetics can in one sense be conceived of as a master switch of cancer biological processes. Recently, there has been growing interest in epigenetic targeting as a promising therapeutic option for cancer [21]. And since cellular metabolism is tightly linked to epigenetic modifications, it is again not surprising that metformin as a modulator of cellular metabolism may also possess significant epigenetic effects mainly via histone modification (**Figure 7**), which in turn is another avenue whereby metformin may exert its anti-cancer effects [9].

2.6 Metformin apoptotic and autophagic effects

Both apoptosis or programmed cell death and autophagy are important catabolic and tumor-suppressive pathways that control cell survival and cell death and are thus increasingly important therapeutic targets in cancer [22]. While apoptosis involves cellular suicide and cell death pathways, autophagy involves recycling and degradation of cellular waste which if maladapted and excessive can also lead to cell death and there is significant cross-talk between these two pathways [23]. In cancer biology, autophagy is cancer suppressive as it facilitates the degradation of oncogenic molecules thus pre-empting the development of cancers, while apoptosis leads to cellular suicide and limits the survival of cancer cells. As a result, defective or inadequate autophagy or apoptosis can both lead to cancer. The complexity of the crosstalk between the apoptosis and autophagy is illustrated in **Figure 8**.

In the case of these pathways, metformin has been shown to promote apoptosis in a variety of cancers via various biological pathways [24] while also promoting autophagy [25] as two other dimensions of its anti-cancer bioactivity.

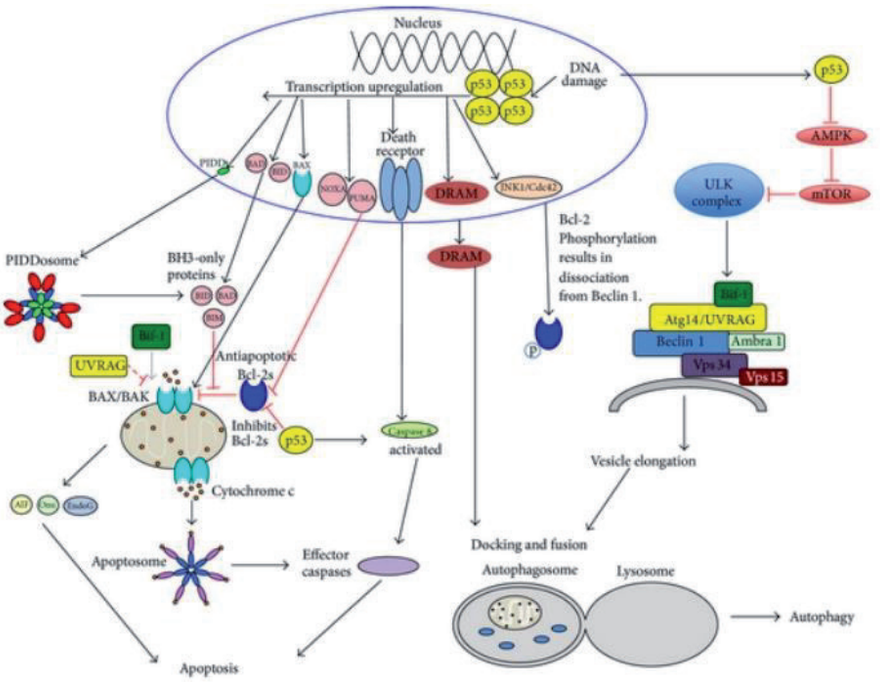

Figure 8.
Complex crosstalk between autophagy and apoptosis pathways. Various proteins involved at the different points of crosstalk are shown and labeled. Lines denote interactions or processes, with solid lines corresponding to intrapathway processes and dashed lines corresponding to inter-pathway connections. Red lines denote inhibitory interactions, while lines with arrows indicate facilitating interactions. Source: [24], Licensed under CC BY 3.0.

2.7 Metformin effects on cancer stem cells

CSCs were only identified in the 1990s, and they have been hypothesized to persist in tumors as a distinct cell population capable of self-renewal and maybe responsible for cancer relapse and metastasis by giving rise to new tumors. These CSCs are also believed to be resistant to traditional chemotherapy and radiation. A complex regulatory network consisting of microRNAs and Wnt/β-catenin, Notch, and Hedgehog signaling pathways control the properties of CSCs. Therefore, the development of specific therapies targeting and its regulatory pathways is another avenue for improved cancer treatments to prevent relapse and metastases, and improve survival [26]. In this regard, metformin has been reported to target CSCs perhaps via blunting of the Warburg effect and consequently down-regulates their growth. In animal studies, it has been found that metformin exposure was associated with a ~ 2-fold reduction in ovarian CSCs and increased in chemotherapy response and translational studies completed as part of a multi-center phase 2 clinical trial was able to demonstrate a 2.4-fold CSC reduction as well as improved survival in ovarian cancer patients [27].

2.8 Metformin's antiangiogenic effects

Angiogenesis is the process where a tumor can induce its own blood supply via neovascularization to enhance its own nutrient source as well as increase its propensity to metastasize. It follows that antiangiogenesis which involves the suppression of vascular supply to tumors may be an effective method of cancer control as initially proposed by Folkman [28]. In this regard, preclinical studies with metformin have reported that it indirectly modulates tumor angiogenesis most likely via metabolic pathways affecting proangiogenic signals. As an example, metformin is known to decrease HIF-1α stability in cancer cells, reducing the expression of HIF-1

targeted genes and thus resulting in smaller tumor vessel size, reduced microvessel density and slower tumor growth [29]. Another murine experiment analyzing angiogenesis in a matrigel plug model found that metformin treatment lead to a decrease in angiogenesis [30].

3. Deployment strategies for metformin in cancer

Metformin can be used tactically under various scenarios against cancer. It can be used as standalone or in combination with other agents for the primary or secondary prevention of cancer [31], as neoadjuvant or adjuvant cancer therapy [32], as maintenance therapy or salvage therapy, or to reduce chemoresistance or enhance radiosensitivity [33] as well as for the reduction of side-effects or complications [34]. Notably, metformin is usually deployed as an adjunct but not as a sole agent except in the case of primary prevention. Since it has such low toxicity and multifaceted mechanisms of actions, it is usually integrated with other treatment agents and modalities under other scenarios besides primary prevention. The key feature of metformin that allows this combinatorial deployment is its low toxicity and its synergism with various other agents and modalities, as it has been demonstrated both *in vitro* and *in vivo*.

3.1 Metformin synergies with other anticancer agents and modalities

Notably, synergisms with metformin has been reported with numerous anticancer agents and modalities including chemotherapy [35], targeted drugs [36], and radiotherapy [37]. In the past ten years alone, metformin synergism with chemotherapies pemetrexed [38], temozolomide [39], cisplatin [40], gemcitabine [41], paclitaxel [42], 5FU [43], vincristine [44] with targeted agents erlotinib against non-small cell lung cancer [45], imatinib against colon cancer [46], gefitinib against bladder cancer [47], trastuzumab against human epidermal growth factor receptor 2 (HER2) positive breast cancer [48], celecoxib against NSCLC [49], regorafenib against liver cancer [50], with everolimus as neuroendocrine cancers [51]; and other anticancer agents such as with nelfinavir against cervical cancer [52], propranolol against breast cancer [53], 2-deoxyglucose against ovarian cancer [54], arsenic trioxide against cholangiocarcinoma [55], and with natural compounds epigallocatechin-3-gallate [56], curcumin [57], berberine [58], resveratrol [59].

What is interesting is that different biological mechanisms may be responsible for the efficacy of metformin's combinatorial effects depending on the specific combination. For example, regulation of lipid synthesis may underlie metformin enhancement of taxanes, pro-apoptotic mechanisms could account for its synergy with cisplatin, AMPK/mTOR signaling maybe significant when combined with hormonal drugs, and suppression of HIF-1, P glycoprotein (p-gp) and multidrug resistance-associated protein 1 (MRP1) expression is thought to be responsible for metformin's synergy with anti-metabolites [60]. In the case of targeted agents such as the epidermal growth factor receptor (EGFR) inhibitor gefitinib against NSCLC where a Chinese study on diabetic NSCLC patients on gefitinib demonstrated significantly improved response rate, disease control rate, median progression free survival (PFS) and median overall survival (OS) compared with patients controls (70.5% vs. 45.7%, $P = 0.017$; 97.7% vs. 80.4%, $P = 0.009$; 19 months vs. 8 months, $P = 0.005$; 32 months vs. 23 months, $P = 0.002$, respectively) [61]. Separately, metformin combination with m-TOR inhibitor everolimus in patients with advanced pancreatic neuroendocrine tumors showed improved median PFS of patients treated with the combination vs. control (median PFS, 20.8 months;

hazard ratio, 0.49; 95% confidence interval (CI), 0.34–0.69; $P < .0001$), suggesting that metformin may sensitize everolimus in these patients [62]. As far as combination with antibody treatments go, a randomized phase II study of metformin plus bevacizumab-based chemotherapy in advanced or metastatic NSCLC patients resulted in a 47% (95% CI, 25%–88%) one-year PFS in patients on metformin, which is much improved over a historical control of 15%. Median overall survival of 15.9 months of metformin treated patients was also improved over control arm of 13.9 months [63]. Furthermore, metformin in combination with immune checkpoint inhibitors (ICI) has received much recent attention as ICI is increasingly being deployed in cancer treatments. A retrospective review of 50 NSCLC patients receiving ICIs as second or third line therapy with or without metformin showed higher overall response rate, disease control, median OS and PFS in the metformin group (41.1 vs. 30.7%, $P = 0.4$; 70.5 vs. 61.6%, $P = 0.5$; 11.5 vs. 7.6 months, $P = 0.5$ and 4.0 vs. 3.0 months, $P = 0.6$, respectively) [64]. Very recently, several significant trials have been launched to further investigate the role metformin may have in combination with ICI's, including a metformin-nivolumab combination in patients with NSCLC (NCT03048500), a phase I trial investigating the combined effect of metformin and another anti-PD-L1 antibody durvalamab in head and neck squamous cell carcinoma (NCT03618654), a phase I trial of metformin in combination of the anti-PD-1 antibody pembrolizumab in advanced melanoma (NCT03311308), and a phase II trial combining metformin with nivolumab in stage IV colorectal cancer that has not responded to previous treatment (NCT03800602).

The use of metformin under various scenarios against cancer has been best studied clinically for primary prevention and in the neoadjuvant setting and some of the relevant data is summarized below.

3.2 Metformin for primary prevention of cancer

Cancer prevention is the earliest role that metformin was hypothesized to play in the disease as it was Evans' original 2005 retrospective case–control study demonstrating metformin's involvement in reducing cancer risk in T2DM that highlighted its potential for cancer [4]. Subsequently, a confirmative cohort study of T2DM with metformin followed in which the frequency of cancer was significantly lower in patients receiving metformin versus controls who had never received metformin, after adjusting for body mass index, hemoglobin A1C, smoking and the use of other drugs [65], a finding that was subsequently repeatedly confirmed. Indeed, meta-analyses have demonstrated that metformin is associated with a decreased risk of breast, colon, liver, pancreas, prostate, endometrium and lung cancer across meta-analyses [31] suggesting that people with T2DM receiving metformin demonstrate a lower risk and improved outcomes with most common cancers; more specifically one meta-analysis found that metformin-treated T2DM patients had a 31% reduction in the incidence of cancer and a 34% reduction in cancer mortality after adjusting for body mass index [66].

3.3 Metformin in neoadjuvant treatment

Neoadjuvant effects of metformin in combination or alone has been clinically explored in several cancers types. In one study of two hundred eighty-five patients with esophageal adenocarcinoma treated with concurrent chemoradiation followed by esophagectomy, complete remission (CR) was higher in T2DM patients taking metformin (34.5%) compared to those who are not (4.8%, $P = 0.01$) as well as non-diabetic patients who are not on the drug (19.6%, $P = 0.05$) and furthermore the CR rate was found to be related to metformin dose, with ≥1500 mg per day associated

with a higher CR rate [67]. In a separate study of diabetic rectal cancer patients undergoing neoadjuvant chemoradiotherapy, those on metformin experienced better tumor responses ($P = 0.002$), pathologic complete remission ($p = 0.037$), and N downstaging ($P < 0.001$) as well as experienced improved cancer specific survival and lower risk of recurrence [68]. Separately, women with endometrial cancer on neoadjuvant metformin 850 mg twice daily for an average of 20 days between diagnosis and surgery had reduced cell proliferation per Ki-67 expression, compared to the untreated [69]. A similar biomarker based on a "window of opportunity" assessment of metformin 500 mg three times daily for a median duration of 18 days in non-diabetic breast cancer also demonstrated that short-term preoperative metformin resulted in both clinical and cellular changes including a significant decrease in the Ki-67 proliferation index from 36.5 to 33.5% ($P = 0.016$) [70]. Separately and perhaps more significantly, a study involving early-stage breast cancer assessing remission rates after neoadjuvant therapy among metformin vs. non-metformin users found a significant difference in CR of 24% in the metformin group, 8.0% in the non-metformin group, and 16% in the non-diabetic group, with metformin use independently predictive of response (OR 2.95; $P = 0.04$) after adjustment for diabetes, body mass index, age, stage, grade, receptor status, and neoadjuvant chemotherapy use by multivariate logistic regression [71].

4. Systematic reviews and meta-analyses on metformin clinical outcomes in various cancers

Since metformin is so versatile and has been studied in a wide variety of settings from the laboratory to bedside, and since this review is intended to focus on the clinical deployment of metformin, it is thus useful to have a summary perspective of its potential usefulness in cancer by reviewing clinical results as recently meta-analyzed for various cancers.

4.1 Bladder cancer

A review of 9 retrospective cohort studies with 1,270,179 patients did not reveal a benefit from metformin in preventing bladder cancer (Hazard ratio (HR) = 0.82, 95% CI = 0.61–1.09; $P = .17$). However, metformin intake was associated with an improved recurrence-free survival (HR = 0.55, 95% CI = 0.35–0.88; $P = .01$), progression-free survival (HR = 0.70, 95% CI = 0.51–0.96; $P = .03$), as well as cancer-specific survival (HR = 0.57, 95% CI = 0.40–0.81; $P = .002$) [72].

4.2 Breast cancer

There have been a number of studies relating to metformin's effect on biomarkers in breast cancer patients and it has been shown that metformin therapy reduced the levels of insulin, sex hormones and sex hormone-binding globulin, Ki67, caspase-3, p-Akt, obesity, CRP, blood glucose and lipid profile overall [73]. More, in a clinical trial to examine the clinical and biological effects of neoadjuvant metformin on patients with breast cancer, non-diabetic women with untreated breast cancer given 500 mg of metformin three times daily for ≥2 weeks exhibited decreased insulin receptor expression ($P = 0.04$), phosphorylation status of protein kinase B /Akt, extracellular signal-regulated kinase 1/2, AMPK and acetyl coenzyme A carboxylase ($P = 0.0001$, $P < 0.0001$, $P < 0.005$ and $P = 0.02$, respectively) in tumors correlating with decreases in tumor cell proliferation and increases in apoptosis [74]. In T2DM patients with breast cancer, a 2018 meta-analysis of eleven

studies of all-cause mortality found a 45% risk reduction was observed for all-cause mortality (HR = 0.55; 95% CI 0.44–0.70) and concluded that metformin may improve overall survival in this patient subset [75]. Separately in another review, 7 observational studies showed significantly reduced breast cancer risk among T2DM patients on metformin OR = 0.83 (CI 0.71–0.97) [76]. Separately, in a sub-study involving over four hundred diabetic patients in the large phase 3 ALTTL trial of Her2+ breast cancer patients, Her2+ and estrogen receptor positive breast cancer cases on metformin experienced had improved disease free survival, metastasis free disease survival and overall survival over those patients not on metformin over a median of four and a half years [77]. However despite the vast amount of preclinical and epidemiologic data on its benefits in breast cancer, there are no trials in non-diabetic breast cancer patients to date which have unequivocally demonstrated a clinical benefit of metformin.

4.3 Colon cancer

Ng et al. from Singapore found 58 studies that provided incidences of colorectal adenoma and cancer and cancer survival outcomes and found that metformin significantly lowered the risk of colorectal adenoma (RR 0.77, CI 0.67–0.88, $P < 0.001$), advanced adenoma (0.61, CI 0.42–0.88, $P = 0.008$) and colorectal cancer (RR 0.76, CI 0.69–0.84, $P < 0.001$) respectively. Overall cancer survival (HR 0.6, CI 0.53–0.67, $P < 0.001$), even among metastatic cases was also higher among metformin users (HR 0.77, CI 0.68–0.87, $P < 0.001$), and it was concluded that metformin significantly reduces colorectal adenoma and cancer incidence as well as enhanced colorectal cancer survival at all stages [78].

4.4 Endometrial cancer

In 19 studies reviewed in 2017, metformin used reversed atypical endometrial hyperplasia to normal, and decreased cell proliferation from 51.94% (CI = 36.23% to 67.46%) to 34.47% (CI = 18.55% to 52.43%) [79], while separately, a review of seven studies showed that metformin could significantly improve overall survival of in endometrial cancer (HR = 0.61, 95% CI 0.48–0.77, $P < 0.05$) and reduce their recurrence risk (OR = 0.50, 95% CI 0.28–0.92, $P < 0.05$) [80], whereas another review of six retrospective cohorts if 4723 endometrial cancer cases demonstrated that metformin use was associated with a significant reduction in overall mortality in comparison with not using metformin (adjusted HR 0.64, 95% CI 0.45–0.89, $P = 0.009$) irrespective of diabetic status [81], and these results corroborated the improved overall (HR, 0.58; 95% CI, 0.45–0.76; $P = 0.207$) as well as progression free survival (HR, 0.61; 95% CI, 0.49–0.76; $P = 0.768$) found in another review of 6242 patients from fourteen studies [82].

4.5 Lung cancer

An analysis of 13 observational studies found lung cancer incidence to be reduced in diabetic patients on metformin vs. no metformin (RR = 0.89; 95% CI, 0.83–0.96; $P = 0.002$) [83]. A separate meta-analysis found six studies comparing metformin usage and non-metformin usage significantly improved overall survival in diabetic patients with NSCLC [pooled HR =0.87 (0.77–0.99), $P = 0.04$] [84]. Especially noteworthy was an ambitious prospective clinical trial conducted by Marrone et al. which studied non-diabetics with advanced or metastatic NSCLC receiving platinum-based doublet chemotherapy and bevacizumab with or without metformin 1000 mg twice daily followed by maintenance

therapy with bevacizumab and metformin combined or bevacizumab alone and showed a significant clinical benefit in PFS (9.6 vs. 6.7 months) with the addition of metformin [63].

4.6 Pancreas cancer

A review of seventeen studies involving 36791 participants study has evidenced a significant association of metformin adjuvant treatment in pancreas cancer with overall survival benefit (HR = 0.88, 95% CI = 0.80–0.97) especially in Asians, those with early stage disease and those undertaking surgery [85]. In terms of overall survival with metformin use in pancreas cancer, a study of 8 retrospective cohort studies and 2 randomized clinical trials representing 3,042 patients revealed overall survival to be improved with metformin (meta-HR = 0.79; 95% CI: 0.70, 0.92, $P < 0.001$) [86].

4.7 Prostate cancer

In a systematic review involving eleven studies with 877,058 patients, the odds ratio of metformin use for reducing prostate cancer was estimated at 0.89 (95%CI: 0.67–1.17) and it was concluded that metformin consumption reduced the risk of prostate cancer, although the result was not statistically significant [87]. Separately, a review of eight studies on diabetic patients with prostate cancer found no metformin use was associated with an increased risk of cancer recurrence (RR, 1.20; 95% CI, 1.00–1.44) [88], which concurs with another review of eight retrospective cohort studies and one nested-case–control study, metformin was found to be associated with a reduced risk of biochemical recurrence (pHR: 0.82, 95% CI 0.67, 1.01, $P = 0.06$) [89]. Finally, a large review of 30 cohort studies, including 1,660,795 prostate cancer patients revealed that metformin treatment compared with no treatment improved overall, prostate cancer specific, and recurrence free survival (HR = 0.72, 95% CI: 0.59–0.88, $P = 0.001$; HR = 0.78, 95% CI: 0.64–0.94, $P = 0.009$; and HR = 0.60, 95% CI: 0.42–0.87 $P = 0.006$, respectively) [90].

4.8 Ovarian cancer

One review of 13 studies involving ovarian cancer incidence and prognosis revealed metformin use to be associated with a lower incidence (pooled OR 0.76, 95% CI 0.62 to 0.93, $P = 0.008$) as well as improved prognosis (pooled OR 0.55, 95% CI 0.36 to 0.84, $P = 0.006$) [91].

4.9 Other cancers

Metformin is also increasingly studied or planned in less common cancers, such as glioblastoma, thyroid cancer, and non-Hodgkin's lymphoma. The recent study on newly diagnosed glioblastoma showed that temozolomide plus memantine, mefloquine, and metformin are feasible as an adjuvant therapy [92]. One planned phase 1b/2 clinical trial of metformin and chloroquine was recruiting patients with IDH1-mutated or IDH2-mutated solid tumors, including glioma [93]. In another recent retrospective study from Korea, cancer preventative effects of metformin on thyroid cancer were observed in individuals with T2DM on long duration or higher doses of the drug [94]. Separately, a trial in head and neck squamous cell cancer patients revealed metformin to inhibit cancer by enhancing apoptosis, and increasing cellular immune infiltration of the cancer [95]. In non-Hodgkin's lymphoma,

a retrospective analysis of looking at T2DM patients treated with standard therapy found improved progression-free survival and overall survival compared to control not taking metformin [96].

5. Discussion

Any discussion of a therapeutic agent is incomplete without covering its toxicity, side-effects and drug interactions. In this regard, metformin is probably one of the safest drugs in use, especially when compared with standard anti-cancer agents in its context as a potential cancer preventative or therapeutic. With its long history of widespread use, its pharmacokinetics and toxicity profile are well established. The most common side-effect is mild to moderate gastrointestinal discomfort or diarrhea which is usually self-limited and can be minimized if metformin is taken with food, while its most serious side-effect of lactic acidosis usually due to overdose is relatively rare, occurring once per 100,000 years of use or 3 case per 1,000,000 after long term treatment [97]. As in the case of all medications, it should be dispensed carefully in elderly patients and in those with impaired renal, cardiac, and hepatic function. For practical purposes, it needs to be emphasized that metformin as an antidiabetic and as monotherapy does not cause hypoglycemia or weight gain, unlike insulin or sulfonylureas. For cancer, because of its very common use in diabetics, it has practically seen combined use with most oncologic agents in the diabetic cancer patient and remarkably no serious interactions with standard cancer anti-cancer agents have been reported. The minimum toxic dose of metformin is not well defined, but rare case reports of severe toxicity has only been reported after ingestion of 25 to 35 grams of metformin by adults.

A treatment for any condition is ideal if it relatively non-toxic and scientifically well evidenced, as well as low in cost and convenient to administer. Metformin fits all the above criteria. It is apparent from our review that metformin has ample scientific evidence from bench to the bedside as a repurposed drug for cancer. In fact, it is safe to say that it is currently the most well evidenced repurposed drug for cancer. Also, its wide-spread and decades of experience of clinical use and low observed toxicities alone or in combination with other agents, as well as very low cost also marks it as an optimal therapeutic agent. Finally, the versatility it possesses against various cancers and its applicability from prevention to treatment further distinguishes it as an ideal or model repurposed drug for cancer.

Of course, there remains limitations and challenges to metformin's use as an anticancer. The first obstacle we have in translating *in vitro* results of metformin to the clinical arena revolves around its dosage. The usual dosage of metformin in cancer trials is the same range as that prescribed normally for T2DM which is from 1000 mg – 2000 mg per day. Treatment is usually started at the lower dose with dose escalations weekly to the maximum dose which means starting at 500 mg of the immediate release version twice daily or 850 mg of the extended release versions once daily, with 500 mg increments weekly as tolerated, to a maximum of 2000–2550 mg per day for either immediate or extended release versions. It may not be apparent at first glance, but the concentration of metformin at 10–100 microM when used clinically at 1000-2000 mg per day is much less than the concentration of >2–5 mM demonstrated for its anti-cancer effects *in vitro* where metformin was usually experimented at concentrations between 5 to 20 mM, which is 2 000–10 000 times more concentrated than achieved with clinical dosing [98]. Fortunately, many clinical studies still yielded positive results at the much lower metformin in concentrations achieved with clinical dosing, but it may also explain why the

clinical results of metformin in cancer may not be as dramatic as demonstrated in preclinical studies, and why it is never intended to be used as monotherapy for cancer treatment. Related to dosing is a possible dose-dependent effect of metformin on cancer risk [99], which raises the question of attempting higher doses of metformin in future clinical trials of metformin in cancer, this while taking into account that there are no cases of acute metformin overdose leading to death found in which patients with a peak serum metformin concentration is under 50 microg/mL [100]. Beyond dosing, another issue with the literature to date on metformin and cancer is that most of the clinical studies so far are retrospective that mainly involve observations in the T2DM patient population and thus subject to selection bias. However, many cohort studies in the non-diabetic is planned, and despite methodological limitations, it is apparent that the overwhelming evidence so far is in favor of potential benefits and a high benefit to risk and benefit to cost ratios for metformin's application in cancer.

6. Conclusion

As an old repurposed drug, metformin is inexpensive and generic and its research is thus carried out usually without industry support. Despite such challenges, it is heartening that overall preclinical and clinical results is overwhelmingly suggestive of a protective effect from metformin against various stages of a wide spectrum of cancers. Moreover, there are over three hundred registered clinical trials on metformin and cancer internationally as of mid-2020, of which approximately one third are actively recruiting. The trials involve metformin for pre-cancers, early stage as well as metastatic solid tumors, alone or in combination with other interventions including chemotherapy, radiotherapy, hormone therapy, immunotherapy (ICIs), targeted agents, statins, aspirin, doxycycline, nelfinavir, melatonin, disulfiram, vitamin C, diet in diabetics and non-diabetics. We thus look forward for the further establishment of metformin as an ideal repurposed agent for cancer prevention and treatment.

Acronyms and abbreviations

Akt	AKT8 virus oncogene cellular homolog
AMPK	AMP-activated protein kinase
CI	confidence interval
CR	complete remission
CSC	cancer stem cell
CTL	cytotoxic T lymphocytes
EGFR	epidermal growth factor receptor
GPDH	glycerol-3-phosphate dehydrogenase
HER2	human epidermal growth factor receptor 2
HIF	hypoxia inducible factor; HR: hazard ratio
hs-CRP	C-reactive protein
ICI	immune checkpoint inhibitor
JNK	Jun N-terminal kinase
mTOR	mammalian target of rapamycin
MRP-1	multidrug resistance-associated protein 1
Myc	myelocytomatosis oncogene cellular homolog
NAD+/NADH	nicotinamide adenine dinucleotide (oxidized)/nicotinamide adenine dinucleotide + hydrogen

NF-kB	nuclear factor k B
NSCLC	non-small cell lung cancer
OS	overall survival
PD-1	programmed cell death protein 1
PD-L1	programmed death-ligand 1
p-gp	P glycoprotein
PI3K	phosphoinositide 3 kinase
PFS	progression free survival
T2DM	type 2 diabetes mellitus
Treg	regulatory T cell

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