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Review

Antitumor mechanisms of metformin: Signaling, metabolism, immunity and beyond

Jorge Eduardo Duque[1,3], Catalina López[2], Nataly Cruz[1], Ismael Samudio[1*]

[1]Grupo de Terapia Celular y Molecular, Pontificia Universidad Javeriana, Bogotá D.C. **Colombia**.
[2]Grupo de Investigación en Terapia Regenerativa, Universidad de Caldas, Manizales, **Colombia**.
[3]Centro Oncológico de Antioquia, Medellín, **Colombia**.

** samudioi@javeriana.edu.co*

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Abstract

Metformin is a synthetic biguanide first described in the 1920´s as a side product of the synthesis of N,N-dimethylguanidine. Like other related biguanides, metformin displays antihyperglycemic properties, and has become the most widely prescribed oral antidiabetic medicine around the world. Intriguing recent evidence suggests that metformin has chemopreventive and direct antitumor properties, and several ongoing clinical studies around the world are using this agent alone or in combination with chemotherapeutic schemes to determine prospectively its safety and efficacy in the treatment of human cancer. Notably, immune activating effects of metformin have recently been described, and may support a notion put forth in the 1950s that this agent possessed antiviral and antimalarial effects. However, how these effects may contribute to its observed antitumor effects in retrospective studies has not been discussed. Mechanistically, metformin has been shown to activate liver kinase B1 (LKB1) and its downstream target AMP-activated kinase (AMPK). The activation of AMPK has been proposed to mediate metformin´s glucose lowering effect, although recent evidence suggests that this agent can inhibit electron transport in hepatocyte mitochondria resulting in AMPK-independent inhibition of hepatic gluconeogenesis. Likewise, albeit activation of AMPK and the resulting inhibition of the mammalian target of rapamycin (mTOR) signaling have been suggested to mediate the antitumor effects of metformin, AMPK-independent growth inhibitory properties of this agent in tumor cells have also been described. Here we present a brief review of the signaling, metabolic, and immune effects of metformin and discuss how their interplay may orchestrate the antitumor effects of this agent. In addition, we provide the rationale for a compassionate use study of metformin in combination with metronomic chemotherapy.

Key words: metformin, cáncer, AMPK, metabolism, diabetes

Resumen

Mecanismos antitumorales de la metformina: señalización, metabolismo, inmunidad y más allá. La metformina es una biguanida sintética descrita por primera vez en la década de 1920 como un subproducto de la síntesis de N, N-dimethylguanidine. Al igual que otros biguanidos relacionados, la metformina muestra propiedades antihiperglicemiantes, y se ha convertido en el medicamento antidiabético oral más recetado en todo el mundo. Datos recientes sugieren que la metformina tiene propiedades antitumorales y quimiopreventivas, y varios estudios clínicos en curso en todo el mundo están utilizando este agente solo o en combinación con regímenes quimioterápeuticos para determinar de forma prospectiva su seguridad y eficacia en el tratamiento del cáncer humano. En particular, los efectos inmuno-activadores de la metformina se han descrito recientemente, y pueden apoyar una idea presentada en la década de 1950 que este agente posee efectos antivirales y contra la malaria. Sin embargo, cómo estos efectos pueden contribuir a sus efectos antitumorales observados en estudios retrospectivos no se ha discutido. Como mecanismo, se ha demostrado que la metformina activa la quinasa de hígado B1 (LKB1) y su proteína diana, la quinasa activada por AMP (AMPK). La activación de la AMPK se ha propuesto como mediador de la disminución de la glicemia sanguínea en respuesta a la metformina, aunque la evidencia reciente sugiere que este fármaco puede inhibir el transporte de electrones en las mitocondrias de los hepatocitos, provocando la inhibición de gluconeogénesis hepática independientemente de AMPK. Del mismo modo, si bien la activación de la AMPK y la resultante inhibición del blanco de la rapamicina en mamíferos (mTOR) se han sugerido

como mediadores de los efectos antitumorales de metformina, inhibición de crecimiento de células tumorales de manera independiente de AMPK también se ha descrito. Aquí presentamos una breve revisión de los efectos de la metformina en señalización, metabolismo, y sistema inmune y discutimos cómo su interacción puede orquestar los efectos antitumorales de este agente. Además, proveemos el fundamento para un estudio de uso compasivo de la metformina en combinación con quimioterapia metronómica.

Palabras clave: metformina, el cáncer, la AMPK, el metabolismo, la diabetes

Resumo

Mecanismos anti-tumorais da metformina: sinalização, metabolismo, imunidade e além. A metformina é uma biguanida sintética descrita pela primeira vez na década de 1920 como subproduto da síntese de N, N-dimetilguanidina. Como outros biguanidos relacionados, a metformina apresenta propriedades anti-hiperglicémicas, e tem-se tornado o medicamento anti-diabético oral mais prescrito no mundo. Intrigante dados recentes sugerem que a metformina tem propriedades anti-tumorais e quimio-preventivas, e vários estudos clínicos em todo o mundo estão usando esse agente sozinho ou em combinação com regimes de quimioterapia para determinar prospectivamente sua segurança e eficácia no tratamento do câncer humano. Em particular, os efeitos imuno-ativadores da metformina tem sido descritos recentemente, e pode apoiar uma idéia apresentada na década de 1950 que este agente tem efeitos anti-virais e contra a malária. No entanto, como esses efeitos podem contribuir para seus efeitos anti-tumorais observados em estudos retrospectivos, ainda não foi discutido. Como mecanismo foi demonstrado que a metformina ativa a quinase do fígado B1 (LKB1) e sua proteína diana, a quinase activada pela AMP (AMPK). A ativação da AMPK tem sido proposta como um mediador de redução da glicemia sanguínea em resposta à glicose com metformina, embora evidências recentes sugiram que este medicamento pode inibir o transporte de elétrons nas mitocôndrias dos hepatócitos, resultando na inibição da gliconeogênese hepática, independentemente de AMPK. Da mesma forma, embora a ativação da AMPK e a inibição resultante do alvo da rapamicina em mamíferos (mTOR) têm sido sugeridos como mediadores dos efeitos anti-tumorais de metformina, a inibição do crescimento de células tumorais independentemente da AMPK, também tem sido descrita. Aqui se apresenta uma breve revisão dos efeitos da metformina na sinalização, metabolismo e sistema imunológico, e se discute como a sua interação pode orquestrar os efeitos antitumorais deste agente. Além disso, se fornece a base para um estudo de uso compassivo da metformina em combinação com a quimioterapia metronómica.

Palavras-chave: metformina, o câncer, a AMPK, o metabolismo, a diabetes

Experimental and clinical evidence of antitumor effects of metformin

The first evidence of the potential antineoplasic utility of biguanides was reported by Dilman and Anisimov in 1979 when they demonstrated that phenformin potentiated the antitumor effect of cyclophosphamide on transplantable squamous cell cervical carcinoma, hepatoma-22a and Lewis lung tumors (1). Interestingly, Dilman and Anisimov also observed in a separate study that phenformin alone inhibited spontaneous carcinogenesis in female C3H/Sn mice (2) suggesting the intriguing possibility that in addition to chemosensitizing properties, the biguanides possessed chemopreventive activity. Additional support for this chemopreventive activity was reported in 2001 when it was demonstrated that metformin completely prevented N-nitrosobis-(2-oxopropyl)amine induced pancreatic adenocarcinomas in high fat-fed hamsters (3). Subsequently, in 2005 Anisimov reported that chronic administration of metformin to female transgenic HER-2/neu mice significantly reduced the number and size of mammary adenocarcinomas (4), suggesting that metformin could also antagonize oncogene driven tumor formation, and in that same year a group from the University of Dundee was the first to report in a retrospective case-control study of type II diabetic patients, that metformin therapy was associated with a significantly reduced risk of developing all types of cancer (5). In vitro mechanistic studies by Zakikhani M et al. in 2006 (6) demonstrated that metformin could antagonize the growth of breast cancer cells via AMP activated kinase (AMPK) signaling, and in 2007 it was reported that this agent induced cell cycle arrest as well as mitochondria dependent apoptosis in glioma cells, both of these events mediated in part by AMPK (7). Additional studies suggested that the effects of metformin did not require an intact p53 signalling pathway (8), and that AMPK-independent mechanisms of cell cycle arrest may also be operational in vitro in inhibiting the growth of prostate cancer xenografts (9). In the face of this preponderance of clinical and experimental evidence, an editorial in the Journal of Clinical Oncology published by Pamela Goodwin in 2009 proposed the use of metformin in the adjuvant treatment of breast cancer (10), mainly citing the drug's ability to reduce hyperinsulinemia (11) which she had reported to be a negative prognostic factor for recurrence (12). It is worth mentioning that the reduction in insulin levels – and the associated decrease in IGF-1 signalling – was also proposed to be a mechanism of action in the studies of Anisimov et al. (4).

Still, the first evidence of the safety and efficacy of metformin as an adjuvant in the treatment of breast cancer was reported by the breast medical oncology group at MD Anderson Cancer Center in a retrospective study of 68 diabetic patients taking metformin, 87 diabetic patients not taking metformin, and 2,374 nondiabetic patients (13). The results of this study demonstrated that diabetic patients with breast cancer receiving metformin and neoadjuvant therapy had a higher pathological response rate than diabetic patients not receiving this agent. Importantly, the use of metformin was not associated with adverse effects in cancer patients receiving chemotherapy. More recent evidence suggests that in vitro metformin can antagonize the growth of chemotherapy-resistant breast cancer initiating cells (14), a finding that indicates the potential utility of this agent in the treatment of relapsed, refractory breast cancer patients. Albeit the clinical evidence favors the use of metformin as an adjuvant in breast cancer treatment, Stanosz S reported that pharmacological treatment with metformin in combination with hormonal agents in young women with well-defined endometrial carcinoma Stage I results in complete remission of the disease after a 6 month treatment and two-year follow up (15). Moreover, the findings of the University of Dundee study (5), and more recently the Zwolle Outpatient Diabetes project Integrating Available Care (ZODIAC) study in the Netherlands (16), suggest that metformin affords chemoprotection against all types of cancer in type II diabetics. In addition, a retrospective study by a group from the University of Washington School of medicine reported that metformin reduces the risk of prostate cancer in Caucasian men (17), and Li D et al. reported that the use of this biguanide was associated with reduced risk of pancreatic cancer in diabetic patients (18). In support of the potential chemopreventive and chemotherapeutic activity of metformin against various types of tumors, this agent has been shown to induce apoptosis of pancreatic cancer cells (19, 20), prevent the growth of human pancreatic cancer xenografts as a single agent (21), and prevent the growth of ovarian (22), prostate (9), and endometrial cancer cells (23) alone and in combination with chemotherapy. In addition, here we present evidence that suggests metformin can also decrease de number of viable leukemia cells in culture (**Figure 1**). Taken together the above results suggest that metformin can 1) antagonize the onset of cancer, 2) im-

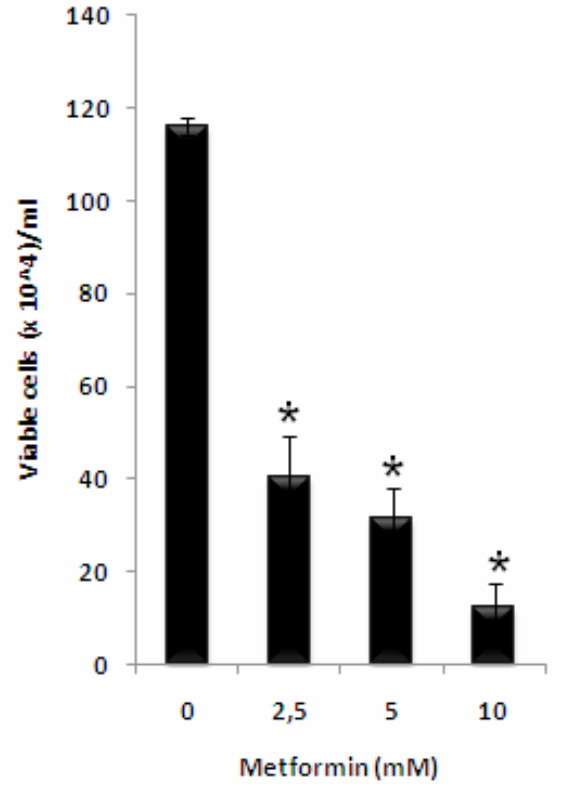

Figure 1. Metformin reduces the number of viable leukemic cells in culture. OCI-AML3 leukemic cells were seeded at a density of 2.5 x 10^5 cells/milliliter in RPMI medium supplemented with 10% fetal bovine serum and treated with increasing concentrations of metformin (kind gift from Michael Andreeff, MD Anderson Cancer Center, Houston USA) for 48 h. Viable cells per milliliter were determined by trypan blue staining in a Neubauer chamber. * = *p<0.0001*

prove the outcomes of traditional chemotherapeutic strategies, and 3) directly inhibit the growth of solid and hematopoietic tumor cells in culture.

Direct and indirect antitumor mechanisms of metformin action

The pioneering work of Anisimov suggested that the mechanism of action of metformin involved downregulation of the insulin/insulin-like growth factor axis (4), a mechanism that has been observed in type II diabetic patients (24) and women affected with polycystic ovary syndrome (PCOS), (25). Nonetheless, more recent experimental evidence has focused on the ability of this biguanide to activate AMPK via the tumor suppressor LKB1 (26), a tumor suppressor kinase whose inactivation leads to Peutz-Jeghers syndrome, a genetic condition characterized by colorectal polyps and predisposition to malignant tumors of various tissues (27). The activation of AMPK, the energy sensor of the cells, results in increased oxidative metabolism and reduced anabolism – reduced lipid synthesis, protein synthesis, etc (28). In addition to direct phosphorylation effects on key metabolic targets like acetyl CoA carboxilase (ACC – committed step in fatty acid synthesis) and phosphofructokinase 2 (PKF2 – master regulator of glycolysis) (29), activation of AMPK also leads to inhibition of the mammalian target of rapamycin (mTOR) (30) which in turn can decrease signaling through the kinase Akt, and reduce the efficiency of protein synthesis via decreased phosphorylation of mTOR targets 4EBP-1 and S6K, essential components of the cap-dependent translation machinery (31, 32). The inhibition of cap dependent translation in response to metformin (33) may in decreased expression of the oncogene Her2 (34) and the cell cycle protein cyclin D1 (9), illustrating a potential avenue by which this agent can modulate signaling and cell cycle effects.

It is important to mention that there are AMPK independent antitumor effects of metformin action such as the Rag GTPase-dependent inhibition of mTOR (35), and the growth inhibition of AMPK silenced ovarian cancer cells (22). In fact, nearly 10 years ago Owen MR et al. described that metformin could inhibit mitochondrial oxidation of complex I dependent substrates in hepatocytes, and this effect could also be observed in isolated mitochondria (36). This inhibition of complex I may contribute to activation of AMPK due to the decrease in oxidative phosphorylation capacity and the subsequent decrease in the ATP/AMP ratio. Moreover, this phenomenon may also account for the occasionally observed lactic acidosis in response to high doses of metformin (37), since pyruvate is now converted to lactate rather than being converted to acetyl CoA in the mitochondria. Indeed, it has been demonstrated that the inhibition of hepatic gluconeogenesis in response to metformin is an AMPK-independent consequence of decreased intracellular ATP levels (38), suggesting that the pleiotropic effects of this agent could be a result of a targeted effect on electron transport in the mitochondria. This effect is most intriguing in light of recent observations demonstrating that the inhibition of electron transport in cancer cells is a lethal insult (39-41), not because of an ensuing energetic catastrophe – cancer cells derive most of their ATP from glycolysis, but because the accumulation of NADH in the mitochondrial matrix inhibits the Krebs cycle and its associated anaplerotic reactions that support the generation of biomass (42). In addition, it has been suggested that electron transport, uncoupled from oxidative phosphorylation, antagonizes the onset of apoptosis in tumor cells (42, 43), supporting the hypothesis that the chemotherapeutic effects of metformin may be the result of its ability to inhibit mitochondrial complex I.

It is also important to consider that immune modulating effects – originally proposed in the 1950s by the Philippine physician Eusebio Garcia (44) – may be an important component of the antitumor effects of this biguanide. A recent thought provoking report demonstrates that metformin can increase memory CD8 T cells in wild-type mice, and in consequence significantly improve the efficacy of an experimental anti-cancer vaccine (45). Mechanistically, this report suggested that increased fatty acid oxidation mediated the generation of CD8 T cells, supporting the notion that activation of AMPK and subsequent inhibition of ACC and malonyl CoA production, promotes fatty acid oxidation. However, this notion is incongruent with the observation that metformin inhibits electron transport in hepatocytes and hepatocyte mitochondria, and it is thus intriguing to hypothesize that metformin modulates tissue specific responses in mitochondrial metabolism – inhibition of electron transport in hepatocytes vs. promotion of fatty acid oxidation in lymphocytes. Regardless, the mechanism the generation of memory CD8 T cells could be a critical component of the chemotherapeutic and chemopreventive action of metformin. Lastly, Ropelle ER et al. have demonstrated that hypothalamic AMPK activation in response to metformin reverses cancer anorexia in tumor bearing rats by inhibiting the production of proinflammatory molecules and controlling the neuropeptide expression in the hypothalamus (46), suggesting another potential benefit of the use of this biguanide as an adjuvant in cancer treatment that warrants further clinical exploration. Taken together, the above observations indicate that the beneficial effects of metformin as an adjuvant in cancer treatment may be orchestrated via

multiple – AMPK–dependent and –independent – mechanisms that could antagonize tumor initiation and/or progression, decrease cancer anorexia, and improve antitumor immunity.

Concluding remarks and therapeutic considerations

Metformin is a safe and effective antidiabetic drug with a potential new indication for the management and chemoprevention of cancer. The evidence presented here suggests that metformin displays single agent efficacy, at least in the setting of chemoprevention, and that it combines favorably with chemotherapy to provide a therapeutic benefit for cancer patients. Figure 2 illustrates the potential mechanisms that ought to be taken into consideration when planning a therapeutic strategy that incorporates metformin. In particular, the inhibition of oxidative phosphorylation by metformin is intriguing since it may account for the activation of AMPK and the sensitization of cancer cells to chemotherapy. Moreover, the activation of AMPK – whether via LKB1 or decreased ATP/AMP ratio – may antagonize cancer cachexia and promote the generation of memory CD8 T lymphocytes to combat malignant cells. These last two effects support the notion that reduced intensity chemotherapeutic schemes, including metronomic chemotherapy regimens, may be effective in combination with metformin since they would produce less immunosuppression and collateral gastrointestinal damage and thus will not antagonize antitumor immunity mechanisms and nutritional status. Even more, recent evidence suggests that metronomic chemotherapy enhances the effects of antitumor vaccines by decreasing circulating Treg cells (47). Since Treg cells can suppress CD8 T cell-mediated immunity it is tempting to speculate that metronomic chemotherapy may interact favorably with metformin to promote better immune control of tumor growth.

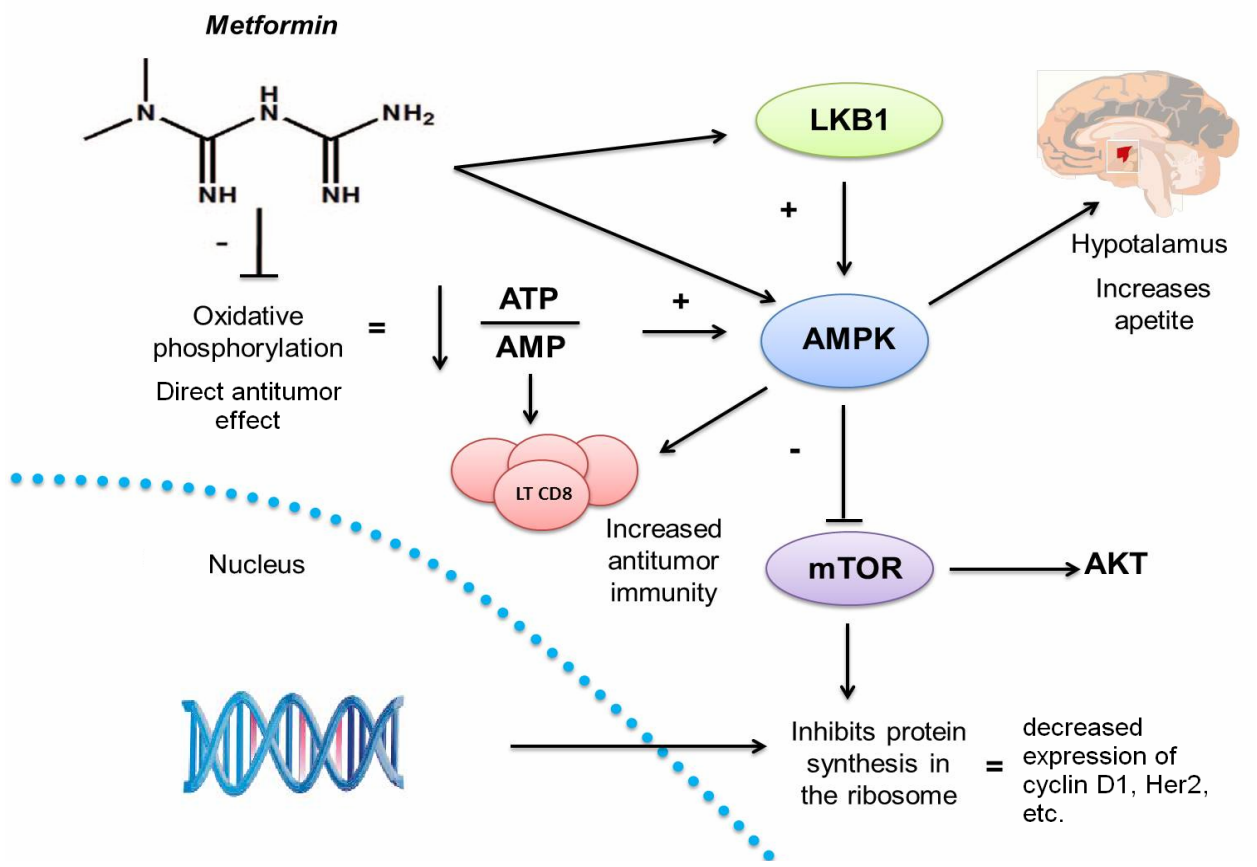

Figure 2. Antitumor mechanisms of metformin action. Metformin may activate AMPK via two separate mechanisms, the inhibition of oxidative phosphorylation/electron transport and subsequent decrease in the ATP/AMP ratio and/or the direct activation of LKB1. In addition to the inhibitory effects on protein synthesis – via inhibition of mTOR – the activation of AMPK may promote the generation of memory CD8 T lymphocytes and the suppression of cancer cachexia signals in the hypothalamus. The inhibition of electron transport may be a lethal insult to cancer cells.

At the time of writing, a search in *ClinicalTrials.gov* yielded only eight open, actively recruiting studies in North America evaluating the efficacy and/or safety of treating cancer patients with metformin. Five of these studies are aimed at breast cancer patients, two are aimed at patients with advanced and metastatic or unresectable tumors, and one is recruiting prostate cancer patients. The prostate cancer study and two of the breast studies use metformin as a single agent prior to surgery to evaluate molecular correlates of response (immunohistochemistry for cell cycle proteins, and proliferation markers) to metformin as a single agent, while the studies in advanced and metastatic or unresectable tumors evaluates the safety of combining this agent with tyrosine kinase or mTOR inhibitors. Why combine metformin, which inhibits mTOR signaling on its own, with an mTOR inhibitor which causes immunosuppression? Why combine metformin with a kinase inhibitor that may cause immunosuppression? Traditional chemotherapeutic regimens do not take into account the damage that they do to the immune system and yet they continue to be a mainstay of cancer therapy. But what if immune sparing chemotherapeutic regimens could be utilized in combination with metformin? To this end, a compassionate use study of metformin in combination with metronomic chemotherapy has been initiated in Colombia based on the notions 1) that metronomic chemotherapy does not cause immunosuppression and may enhance the immune activating antitumor effects of metformin by decreasing Treg cells; 2) the chemosensitizing effects of metformin will potentiate the antitumor effects of metronomic chemotherapy; 3) the low toxicity of metformin allows for its use in patients with a low performance status, excluding those that are prone to lactic acidosis due to kidney malfunction or other condition; 4) metformin has the potential to counteract cancer anorexia; and 5) the low monetary cost of metformin and the low monetary cost of metronomic chemotherapy allows patients to cover the costs of their own treatment.

The above therapeutic considerations, in addition the low economic cost of metformin and metronomic chemotherapeutic regimens, warrant the initiation and support of additional clinical studies that evaluate the efficacy of metformin in patient populations that are not eligible for standard chemotherapeutic schemes. If the results of the compassionate use study in Colombia suggest a therapeutic benefit of metformin in combination with metronomic chemotherapy, this may represent a novel paradigm for the treatment of human malignancies that reduces not only the initial cost of treatment, but the cost of treatment related complications that place such a heavy burden on health systems around the world.

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Conflict of interests

The authors do not have conflict of interests to declare.

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