Investigating Metformin for Cancer Prevention and Treatment: The End of the Beginning

Michael N. Pollak

ABSTRACT

Laboratory research and pharmacoepidemiology are providing converging evidence that the widely used antidiabetic drug metformin has antineoplastic activity, but there are caveats. Although population studies suggest that metformin exposure is associated with reduced cancer risk and/or improved prognosis, these data are mostly retrospective and nonrandomized. Laboratory models show antineoplastic activity, but metformin concentrations used in many experiments exceed those achieved with conventional doses used for diabetes treatment. Ongoing translational research should be useful in guiding design of clinical trials, not only to evaluate metformin at conventional antidiabetic doses, where reduction of elevated insulin levels may contribute to antineoplastic activity for certain subsets of patients, but also to explore more aggressive dosing of biguanides, which may lead to reprogramming of energy metabolism in a manner that could provide important opportunities for synthetic lethality through rational drug combinations or in the context of genetic lesions associated with hypersensitivity to energetic stress.

Significance: There are tantalizing clues that justify the investigation of antineoplastic activities of biguanides. The complexity of their biologic effects requires further translational research to guide clinical trial design.

INTRODUCTION

Investigations of botanical preparations used for medicinal purposes in medieval Europe (and also in traditional Chinese medicines) led to the recognition of the metabolic effects of biguanides, and subsequently to the widespread use of metformin in the treatment of type II diabetes (1, 2). Interest in the potential relevance of biguanides to neoplastic disease was stimulated by a seminal 2005 report (3) describing reduced cancer burden in diabetic patients treated with metformin as compared with those treated with other diabetes therapies. This led not only to further research in pharmacoepidemiology, but also to laboratory studies. To the surprise of many investigators, biguanides were shown to have cell-autonomous antineoplastic activity in many *in vitro* models, starting with a report in 2006 (4). In retrospect, however, the mechanisms uncovered by many of these laboratory studies may differ from those that may operate in diabetics treated with metformin, as the exposure levels differ significantly. During the last 5 years, interest in this field has grown exponentially, and has been reviewed extensively (5–10). Here, emphasis will be on the pivotal studies, the most recent studies, and current controversies.

As the number of population studies has increased, inconsistencies have appeared, and there is increasing attention to statistical methodology, to confounding factors, and to the possibility that if cancer burden is reduced by metformin, this effect may be confined to certain subpopulations and/or to certain kinds of cancer. The applicability of findings concerning possible effects of metformin on cancer risk in cohorts of type II diabetic subjects [who are known to have increased cancer risk relative to the general population (11)] to metabolically normal subjects has neither been established nor ruled out.

Meanwhile, metformin has been studied in dozens of models of established cancers and also in experimental carcinogenesis systems. This work has not only shown antineoplastic activity, but also has suggested several plausible mechanisms of action. However, relatively little attention has been given to pharmacokinetics and to the drug exposures used experimentally relative to those achievable clinically. This involves not only the issues of whole-organism drug distribution but also the cellular pharmacokinetics of drug uptake (12–14).

Investigation of potential indications of metformin in oncology is appealing because the drug is inexpensive, relatively safe, and seems to involve, at least in part, modulation of energy metabolism, which is a cancer research theme that is attracting increasing interest (15–20). Furthermore, there is interest in possible "antiaging" or "calorie restriction

Author's Affiliation: Department of Oncology, McGill University; Segal Cancer Centre, Lady Davis Institute for Medical Research, Jewish General Hospital, Montreal, Quebec, Canada

Corresponding Author: Michael N. Pollak, McGill University, Jewish General Hospital, 3755 Cote-Sainte-Catherine Road, Montreal, Quebec H3T 1E2, Canada. Phone: 514-340-8222; Fax: 1-514-340-8600; E-mail: michael.pollak@mcgill.ca

mimetic" activities of metformin, which may involve mechanisms also relevant to antineoplastic effects (21–23).

In view of its status as a generic compound with widespread availability, investigations of metformin have not been coordinated centrally as is usually the case with a novel drug candidate. As the field of study matures, one research direction is based on the premise that metformin, as used in diabetes, is not necessarily the optimum biguanide regimen for oncologic indications in terms of pharmacokinetics, and that it is best regarded as a lead compound requiring optimization before clinical investigation. Another research goal, which is further advanced, is to evaluate metformin itself at conventional antidiabetic doses for possible use in oncology, particularly for indications that may require long-term administration, where its extensive safety record is of paramount importance. These lines of investigation are not mutually exclusive, and both may be regarded as interesting examples of "repurposing" research, in which novel indications and mechanisms of action of an existing class of drugs are examined (24).

PHARMACOEPIDEMIOLOGY: HYPOTHESIS-GENERATING CLUES

Rarely are data concerning cancer incidence and outcome among populations already exposed to a drug candidate able to contribute to the rationale for further research and development, but this is precisely the situation that has arisen with metformin. Many investigators have used population registries to examine cancer risk among diabetic subjects who were or were not treated with metformin. Other studies are confined to subjects known to have both diabetes and cancer and have attempted to determine whether the use of metformin as diabetes treatment influences cancer prognosis (as distinct from risk). The majority of these studies are retrospective in nature, and the use of metformin is not randomized (except for rare cases, where use of metformin may have been allocated randomly in the context of a clinical trial regarding diabetes treatment). These data are more complex to interpret than one might initially expect. Among other issues, there is evidence that diagnosis of diabetes may influence probability of cancer detection (25), and it is possible that the decision to use metformin for diabetes treatment (rather than other agents such as insulin) is influenced by clinical and metabolic factors that may also influence cancer risk or cancer prognosis, leading to a situation in which metformin use may be associated with reduced cancer burden, but not responsible for it.

Recent studies (reviewed in refs. 26, 27) suggesting reduced cancer risk (e.g., refs. 28, 29) or improved outcome (e.g., refs. 30–35) associated with metformin exposure must be balanced against others that do not show such associations (e.g., refs. 36–39). Some studies suggest unexpected variables that might modify the effects of metformin, including pharmacoepidemiologic evidence that exposure to both a statin drug and metformin is necessary for an important antineoplastic effect to be observed (40) and laboratory evidence that administration of proton pump inhibitors limits cellular uptake of metformin (41).

Taken together, the retrospective research is best regarded as hypothesis-generating rather than definitive. It clearly identifies exciting possibilities and contributes to the justification for further population, translational, and laboratory studies. The extent to which nonrandomized studies concerning influence of metformin use on cancer burden in diabetics should contribute to the rationale for clinical trials in nondiabetics is a point for discussion, but data concerning cancer incidence in the Diabetes Prevention Trial (42), or other cohorts in which exposure to long-term metformin was randomized, will certainly be useful in this regard.

LABORATORY STUDIES: PLAUSIBLE MECHANISMS

Mitochondrial Site of Action

Despite its widespread use in treatment of type II diabetes, details of the mechanisms of action of metformin in this disease were only recently elucidated (43–45), and gaps in knowledge remain. These mechanisms are likely relevant to its activity in cancer prevention and treatment. Many investigators now believe that the fundamental mechanism of action of biguanides involves inhibition of mitochondrial oxidative phosphorylation, and more specifically, that metformin acts to inhibit respiratory complex I (46–50). However, although there has been major progress in understanding complex I (e.g., ref. 51, 52), there are no direct data to show that biguanides directly bind to complex I components, and therefore indirect cellular mechanisms by which biguanides could act to limit oxidative phosphorylation must also be considered. There are many precedents for natural products with growth inhibitory activity to act on mitochondria (53). However, it is of interest to ask why biguanides are not as toxic as well-known poisons that inhibit oxidative phosphorylation, such as cyanide. One proposal (47) is that biguanides require active transport into mitochondria, and that as they reduce mitochondrial function, this transport is inhibited, leading to a dynamic equilibrium, which limits the magnitude of their effect—but the clarification of this point will require a deeper understanding of the precise molecular target of biguanides.

The mitochondrial actions of biguanides may have direct and/or indirect consequences relevant to cancer biology. If metformin exposure is adequate *in vivo*, transformed cells will be subjected to energetic stress. This will have a variety of consequences, some of which may be therapeutically useful, as discussed below and illustrated in Fig. 1. Indirect effects that arise as a consequence of direct metformin actions on host organs must also be considered. Perhaps the most obvious indirect effect is a consequence of metformin action on the liver. Pharmacokinetic factors favor activity in the liver because it is exposed to relatively high drug concentrations via the portal circulation following oral administration, and because hepatocytes express high levels of cell surface transport molecules, such as OCT1, that facilitate metformin entry. Metformin-induced hepatic energy stress leads to decreased gluconeogenesis (43–45). This reduces hepatic energy requirements, lowers hepatic glucose output and circulating glucose levels (provided they are elevated at baseline), and secondarily lowers insulin levels, provided that hyperinsulinemia is present at baseline. This may lead to an antiproliferative action in the specific setting of insulin-responsive cancers in hyperinsulinemic patients (5).

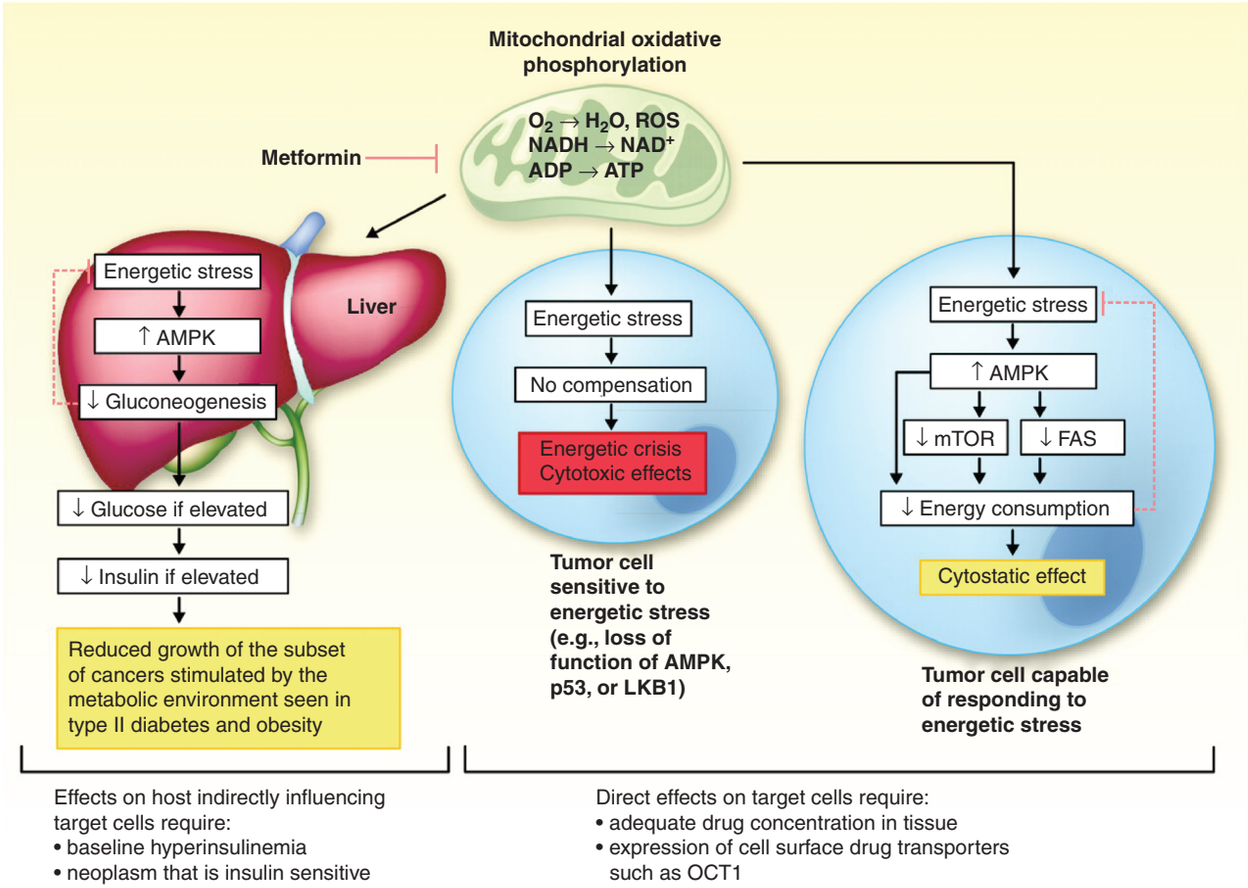

Figure 1. A simplified view of proposed antineoplastic mechanisms of action of biguanides. The initial site of action is likely in the mitochondria, where biguanides interfere with oxidative phosphorylation via a poorly characterized interaction with respiratory complex I, resulting in reduced ATP production and energetic stress. It is known that conventional dosing of metformin is sufficient for this process to occur in the liver, because hepatocytes express at a higher level the proteins that import the drug, and because following oral ingestion, the portal circulation has high levels of the drug relative to the systemic circulation. As shown on the left, this can result in an indirect cytostatic effect on certain tumors, even if metformin does not accumulate in neoplastic tissue. This process involves the suppression of liver gluconeogenesis due to hepatocyte energy stress, leading to declines in circulating insulin and glucose levels (provided that these are elevated at baseline), which in turn may inhibit the growth of the subset of cancers that thrive in a hyperinsulinemic and hyperglycemic environment. Shown in the center is a separate process that may occur if adequate drug levels are achieved in tumor cells, a possibility which has not yet been examined in detail clinically. In this setting, those tumor cells that have deficits in ability to cope with energetic stress may undergo an energetic crisis leading to death. Finally, shown on the right, if adequate drug levels are achieved in cancer cells that have intact mechanisms to cope with energetic stress, biguanides are expected to modulate signaling pathways in a manner that will result in reduced cellular energy consumption. This would be expected to have an important antiproliferative effect, but might also favor cell survival under certain conditions. As discussed in the text and in Table 1, these mechanisms suggest opportunities for rational combination therapies of biguanides with other agents. Metformin is a suitable agent for clinical trials related to the "insulin reduction" mechanism, but biologically significant declines may be confined to patients with hyperinsulinemia at baseline. It remains to be determined if orally administered metformin at conventional antidiabetic doses achieves sufficient drug levels in neoplastic tissue to allow for clinical evaluation of the proposed "direct" mechanisms of action, or if this will require the development of novel biguanide formulations designed to minimize adverse effects (at least for short-term administration) while achieving adequate neoplastic tissue exposure.

Cellular Consequences of Inhibition of Oxidative Phosphorylation by Biguanides

There has been deserved emphasis in the cancer energetics literature on the Warburg effect (15), which involves increased glycolysis in neoplastic tissue. However, cancer cells, like their normal counterparts, require mitochondria for their contribution to ATP production as well as other critical metabolic functions (18). What, then, are the consequences to transformed cells of inhibition of oxidative phosphorylation by biguanides? Obviously, ATP production declines, as does oxygen consumption. The reduction in ATP level triggers activation of the cellular energy regulator AMP-activated protein kinase (AMPK) (53). This leads to reprogramming of cellular energy metabolism in a manner intended to restore ATP levels. In the setting of biguanide-induced limitations on oxidative phosphorylation, this involves increased glucose uptake and glycolysis and also downregulation of the processes that consume ATP. Although the extent to which biguanides accumulate in neoplastic tissue in patients has not been established, if sustained levels sufficient to limit ATP production are achieved, one would expect that an antiproliferative

"energy-saving" phenotype would be induced, as originally observed *in vitro* (4). This phenotype would involve downregulation of energy-consuming processes, such as protein synthesis via mTOR inhibition (4, 54–56) and fatty acid synthesis via reduction in fatty acid synthase expression (57, 58). A transformed cell adopting an "energy-saving" phenotype is unlikely to behave in an aggressive fashion, so a beneficial cytostatic effect is plausible. However, in keeping with its evolutionary role, the activation of AMPK in certain contexts enhances survival (59–61). This may or may not have adverse clinical implications. As there is precedent for a novel therapy to have adverse or beneficial effects depending on context (62), this issue merits attention.

The tumor suppressor gene *LKB1* participates in the functioning of AMPK, and is nonfunctional in tumors associated with Peutz–Jeghers syndrome, as well as in subsets of lung and endometrial cancers (56). Furthermore, there is early evidence (63) that some human breast cancers have lower activation of AMPK than adjacent normal tissue. What then would be the consequence of exposure of cancer cells with defects in AMPK signaling to metformin? Under often-used but nonphysiologic tissue culture conditions characterized by high glucose levels near 20 mmol/L, metformin has little effect on cells that are defective in AMPK signaling (4), suggesting that the antiproliferative action of metformin under these conditions is indeed AMPK dependent. Under these conditions, energetic stress associated with inhibition of oxidative phosphorylation may be attenuated by compensatory high rates of glycolysis. However, at more physiologic glucose levels, cells that are defective in AMPK signaling are actually hypersensitive to metformin (64). This can be interpreted in an evolutionary context: AMPK signaling evolved to enhance survival under conditions of energetic stress, even if this requires a reduction in proliferation. When mitochondrial ATP production is reduced by metformin, absence of functional AMPK or its downstream effectors required for proliferation inhibition (e.g., p53; ref. 65) implies energy deficiency without a compensatory reduction in energy consumption, resulting in an energy crisis and cell death. This line of research implies that effects of biguanides are likely to vary with metabolic and genetic characteristics of tumors (64). Of special interest is the possibility of "synthetic lethality," whereby a biguanide has a cytotoxic effect only in the context of a genetic defect [such as loss of p53 (65) and/or LKB1 (64)] that is present in the cancer, but not in the host, raising the possibility of a favorable therapeutic index. If further clinical studies support early clues (66) of heterogeneity between tumors in response to biguanides, it will be important to design definitive clinical trials accordingly and make use of any available predictive biomarkers.

An early report (63) provides evidence that AMPK is often less activated in human cancer tissue than in corresponding normal tissue. This can be interpreted in the context of a tumor suppressor function of AMPK: its activation leads to an energy-saving antiproliferative (but prosurvival) effect, so in neoplastic tissue, there may be selection for rapidly growing clones with decreased AMPK activation—this provides a growth advantage to transformed cells, but also a potential "Achilles heel" that could be therapeutically exploited, as such clones would have reduced tolerance to energetic stress. Thus, although biguanides can act as AMPK activators, they may be more effective antineoplastic agents than compounds that activate AMPK without inducing energetic stress (67).

Many other cellular effects of biguanides have been described. It is likely, but not proven, that these all are ultimately attributable to the primary site of action in the mitochondria. One example of potential relevance to cancer prevention concerns evidence that metformin not only reduces ATP production as a complex I inhibition, but also reduces reactive oxygen species (ROS) production, consistent with the fact that complex I is an important source of ROS (68). This action, in an *in vitro* model, is sufficient to reduce DNA damage and mutation rate, and if confirmed *in vivo*, could account for reduced cancer incidence observed in certain pharmacoepidemiologic studies. There is separate evidence that metformin affects the redox status of the cell by inhibiting NADH consumption in the mitochondria, influencing the tricarboxylic acid cycle (69). Many studies describe additional interesting consequences of metformin exposure, but mechanistic details and clinical relevance remain to be explored. These include effects on stem cells (e.g., refs. 70, 71), microRNAs (e.g., ref. 72), expression of specific genes relevant to neoplasia, such as aromatase (73) or p-glycoprotein (74), and others.

EFFECTS AT THE WHOLE-ORGANISM LEVEL

Systemic effects of metformin in diabetic patients were studied before cellular mechanisms were investigated, but remain incompletely described. There are also important gaps in knowledge concerning systemic effects in nondiabetic subjects. Type II diabetes is characterized by insulin resistance in classic insulin target tissues such as liver, muscle, and fat, leading to hyperglycemia and secondary hyperinsulinemia. Metformin lowers glucose levels if they are elevated, leading to secondary reduction of insulin levels. Diabetologists originally emphasized studies of metformin action in metabolic tissues that control blood glucose, without considering the effect of the drug on "bystander" organs relevant to oncology, such as prostate, breast, or lung, or tumors arising from them. An important point to bear in mind is that effects of metformin are unlikely to be homogeneous across tissues, not only due to higher concentration in the portal circulation than the systemic circulation following oral dosing, but also due to the fact that tissues vary in their expression of the transport molecules required for metformin uptake. Although these transporters play a key role for metformin uptake at drug concentrations achievable *in vivo*, cellular accumulation of other more lipophilic biguanides, such as phenformin, are less dependent on active transport, and therefore may differ greatly from metformin in terms of tissue distribution and have greater antineoplastic activity, as suggested by laboratory studies (75–77).

Among the more important systemic effects of metformin in diabetes are an increase in muscle glucose uptake (78) and suppression of gluconeogenesis [the output of glucose by the liver (43–45)], both of which contribute to a lowering of circulating glucose concentration. When baseline insulin is elevated, this can result in concomitant reduction of insulin secretion.

Hyperinsulinemia has been identified as an adverse prognostic factor and/or risk factor for several common cancers, including breast (79, 80), colon (81, 82), and prostate (83), suggesting that metformin could slow the growth of the subset of tumors that are insulin responsive by lowering insulin levels (5). It is of interest that this action does not require accumulation of metformin in neoplastic tissue (or in the context of prevention applications, in at-risk tissue), as the reduction in insulin level is a consequence of metformin action in classic metformin target tissues such as liver and muscle. There is experimental evidence to support this mechanism of action of metformin (64). Although there is ample evidence that insulin signaling may stimulate the survival and proliferation of a subset of cancers (64, 84, 85), and recent evidence suggests that insulin diverts carbon flux in a manner that favors neoplastic growth (86), it nevertheless remains possible that the association of hyperinsulinemia with poor outcome involves mediators other than insulin itself. Thus, ongoing studies may identify changes in hormones, cytokines, or serum metabolites that influence tumor growth and vary with metformin exposure to a greater effect than insulin.

Declines in fasting insulin level with metformin treatment are seen when hyperinsulinemia is present, but this effect diminishes with lower baseline insulin levels, and it is not clear if any physiologically relevant decline occurs in subjects with baseline levels below 45 pmol/L (87). A recent study showed declines in fasting insulin following 1,500 mg metformin daily from 13.6 ± 5.4 to 10.0 ± 4.8 uIU/mL in subjects with baseline insulin resistance with lesser declines in subjects not insulin resistant at baseline (88). These changes are considerably smaller in magnitude than those seen in preclinical models, in which metformin reduced insulin levels by about 50% in mice with diet-induced hyperinsulinemia, a change sufficient to reduce tumor insulin receptor activation and growth rate (64). A prior therapeutic strategy used a somatostatin analogue to reduce insulin and insulin-like growth factor (IGF)-I levels in the adjuvant treatment of breast cancer. This resulted in statistically significant, but small magnitude, changes in IGF-I and C-peptide in the hypothesized direction, but no clinical benefit after 2 years' exposure (79). However, metformin may represent a more effective pharmacologic strategy than the use of a somatostatin analogue in this context, and this hypothesis is currently under study in a large breast cancer adjuvant therapy trial (89), which may not only clarify the hormonal effects, but also determine if they are correlated with any antineoplastic activity.

Acute and long-term effects of metformin on both fasting and postprandial insulin levels in nondiabetic subjects requires further investigation, as does the hypothesis that baseline insulin level, when combined with tumor characteristics suggesting insulin responsivity [such as the absence of activating phosphoinositide-3 kinase (PI3K) mutations], can be used to define a subpopulation in whom metformin-induced decline in insulin level may be associated with antineoplastic activity.

Another point to consider in this context is the influence on cancer risk and prognosis of the relatively high insulin levels present in people with insulin resistance treated with subcutaneous insulin. Earlier studies (11) raised the possibility of excess cancer burden in this situation, which was consistent in a general sense with studies concluding that endogenous hyperinsulinemia (unrelated to insulin therapy) is associated with poor prognosis (79–83). More recent research (25, 90) concludes that insulin therapy is not associated with an increase in cancer burden, leading to interesting questions concerning exogenous versus endogenous insulin exposure in relation to cancer burden.

Interestingly, androgen deprivation therapy, which is a standard practice for metastatic prostate cancer, raises insulin levels (91, 92), so if metformin lowers the hyperinsulinemia seen in this situation, there will be a strong rationale to examine the benefit of combining metformin with androgen deprivation (Fig. 2). This relates not only to the adverse effect of hyperinsulinemia on prognosis (83) but also to evidence that insulin promotes local androgen synthesis by prostate cancer cells, which is thought to represent a resistance mechanism to castration (93).

Although insulin receptor family tyrosine kinase inhibitors are more effective than metformin (94) in inhibiting activation of the members of this receptor family (insulin receptors, IGF-I receptors, and "hybrid" receptors), this must be balanced against the favorable long-term safety profile of metformin. Interestingly, the use of kinase inhibitors that target these receptors or key downstream signaling nodes is often associated with hyperglycemia, which is usually managed by addition of metformin. Although metformin is prescribed in this context to manage a metabolic complication of the kinase inhibition, care is required to determine if co-administration of metformin contributes to any antineoplastic activity attributed to the inhibitor (5, 95).

Most studies of the systemic effects of metformin that may be relevant to oncology have emphasized the reduction of insulin levels that are seen in the subsets of treated individuals as a candidate mediator, but other whole-organism effects also deserve attention. These include possible effects on other cytokines and growth factors, including adiponectin (66, 96, 97), and immunologic effects (98). Recent evidence (54, 99, 100) confirms that metformin acts as an inhibitor of mTOR and protein translation *in vitro*, but it is unclear to what extent this occurs *in vivo* (in either neoplastic or normal tissues), as drug levels and expression of cell surface transporters may be limiting. The importance of newer indications for mTOR inhibition (101) makes this an important area for investigation, and if mTOR inhibition is documented in clinical trials of metformin, pharmacodynamic studies should clarify if this is secondary to reduced insulin levels and/or to AMPK activation.

It is relevant that a recent study (102) suggests that certain adverse effects of currently used mTOR inhibitors maybe are attributable to upregulation of gluconeogenesis; biguanides have the potential to inhibit mTOR without this disadvantage. The difference relates to the direct inhibition of mTOR (without concomitant energy stress) by currently used mTOR inhibitors, as compared with mTOR inhibition in the setting of biguanide-induced energetic stress (56) or biguanide effects involving a Rag GTPase–dependent mechanism (103). It is important to emphasize, however, that pharmacokinetic factors and integrity of AMPK signaling will influence the extent of biguanide-induced mTOR inhibition in a tissue-specific manner.

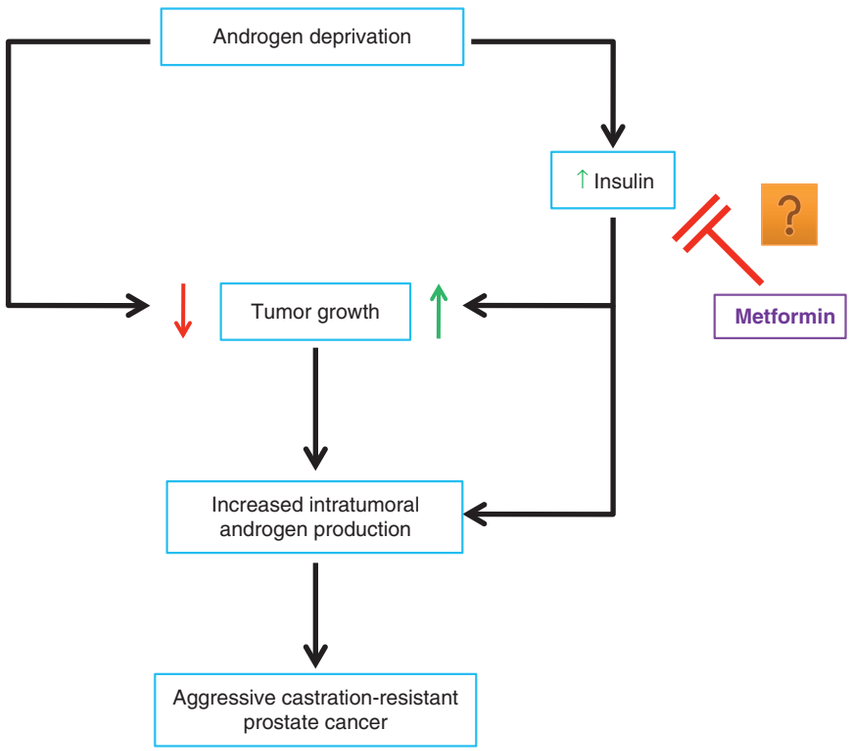

Figure 2. An example of a possible specialized application of metformin in cancer treatment. Many contexts in which metformin may have a use in cancer treatment have been proposed. One example involves use with androgen deprivation therapy in prostate cancer. As discussed in the text, androgen deprivation has obvious benefit for men with metastatic prostate cancer, but this is temporary, and is associated with adverse effects related to androgen deficiency–induced hyperinsulinemia, including increased cardiovascular disease risk. As insulin may directly stimulate neoplastic growth of certain prostate cancers, or upregulate intratumoral testosterone synthesis, the hyperinsulinism of androgen deprivation may also contribute to progression to castration-resistant disease. If ongoing research shows that metformin attenuates the hyperinsulinemia associated with androgen deprivation, combined androgen deprivation and metformin may improve both tolerability and efficacy.

TRANSLATIONAL RESEARCH AND EARLY CLINICAL TRIALS

Many exploratory clinical studies with pharmacodynamic endpoints are underway, and a few have been completed. An early report (104) examined metformin effects on breast cancer cell proliferation in nondiabetic women with operable breast cancer. The design of this study involved comparisons of serum and tissue biomarkers obtained at baseline and following metformin administration. The strength of this study was the fact that tissue specimens at each timepoint were obtained by a similar biopsy procedure, although the study was not placebo-controlled, and in common with most studies, serum sampling did not involve formal fasting and postprandial specimens. As expected for women not hyperinsulinemic at baseline, metformin use was associated with no change in insulin level, but women not assigned to metformin showed an unexpected increase in insulin level between the initial biopsy and surgery. A decline in tumor cell proliferation as estimated by Ki-67 staining was observed with metformin treatment, but the study size was too small to allow for analysis in subpopulations.

It is instructive to compare this study with another (66, 96, 97) of similar design. This study was considerably larger (n = 200), and was carried out in a randomized, placebo-controlled manner—an obvious strength. However, the second tissue sample was obtained at surgery rather than by a second biopsy procedure, and there was some variability in the time between the last metformin dose and obtaining the surgical specimen, factors that could complicate interpretation of findings. Ki-67–estimated proliferation rates increased between biopsy and surgery in placebo-treated women, a finding that is incompletely understood but has been observed in other studies (105, 106). This rise was blunted in women receiving metformin, particularly in subsets defined by high body mass index (BMI), C-peptide, or IGFBP-1. In these subsets, significantly lower Ki-67 index was observed in subjects receiving metformin than placebo. However, in certain subsets metformin administration was associated with a modestly increased proliferation rate. This is unexplained, but the possibility that in some situations, metformin-induced AMPK activation can increase VEGF secretion or metabolically favor survival must be considered (59, 61). In any case, this study suggests that any benefits of metformin may be confined to subpopulations of women defined by tumor or host metabolic characteristics (97). This study also provided preliminary evidence that circulating metformin level is a variable that influences antiproliferative activity. A third similar study (107) was smaller (n = 39), not placebo-controlled, and also compared tissue from needle biopsies with surgical specimens. Nonsignificant declines in insulin level together with significantly increased apoptosis and reduced proliferation were observed. Collectively, these "window-of-opportunity" biomarker trials show alterations in pharmacodynamic endpoints, but do not establish if systemic as compared with local actions of metformin underlie the effects seen. They raise important questions about variables that may modify metformin effects, including BMI, insulin resistance, breast cancer subtype, and drug levels.

The 2 studies that included untreated controls (66, 104) raise the interesting possibility of a perioperative elevation of both insulin levels and cancer cell proliferation. One may speculate that the former could contribute to the latter

and that both might be blunted by metformin in certain subgroups. This deserves study in the general context of the metabolic effects of the perioperative procedures on cancer biology, and more specifically, the hypothesis that metformin may be of particular value when administered in the perioperative period. More specifically, it is conceivable that some patients with cancer may have high perioperative levels of insulin or other cytokines related to routine administration of intravenous glucose perioperatively (regardless of their preoperative levels). This could have negative impacts on cancer outcome by favoring the survival of any insulin-sensitive tumor cells released into the circulation, an effect that could be attenuated by metformin, or simply avoided by minimizing perioperative intravenous glucose load.

A suppressive effect of low-dose (250 mg/d) metformin on aberrant crypt foci in the colon was reported in a short-term trial (108). Although a systemic effect with this dose is unlikely, the observation is consistent with relatively high intraluminal metformin concentrations following oral administration. Indeed, it is of interest that reports in the clinical literature on 18-fluoro-deoxy-glucose positron emission tomography (FDG-PET) have documented increased intestinal glucose uptake in patients receiving metformin (109). Although this was discussed in terms of its significance in diagnostic imaging, it is possible that that the metformin-associated increased FDG-PET signal may represent a pharmacodynamic marker of metformin activation of AMPK in the intestine, leading to increased glucose uptake. AMPK activation by metformin or other agents may simultaneously increase glucose uptake and inhibit proliferation, complicating the use of FDG-PET as a marker of response. The effects of metformin on FDG-PET images may vary with context, as reduction of insulin levels by the drug will tend to reduce glucose uptake by insulin-responsive cancers, an action that would compete with any direct AMPK-stimulated increases in glucose uptake (110).

A final example of a small pilot clinical trial ($n = 22$) using a "window-of-opportunity" design was carried out in men with early prostate cancer (not in the setting of androgen deprivation), with comparison of proliferation of preoperative biopsy specimens and prostatectomy specimens (111). A trend towards a decline in serum prostate-specific antigen was observed, but this did not reach statistical significance. However, a small but significant reduction in proliferation rate was noted. The mechanisms involved require further study, as no significant reduction in insulin was noted, and serum metformin level was lower than that required for *in vitro* activity.

CHALLENGES FOR FUTURE RESEARCH

Many phase II and III trials of metformin are in progress (as of June 2012, the clinicaltrials.gov database lists more than 30). It is beyond the scope of this article to review these individually, but it is worth emphasizing that by incorporating well-designed companion studies involving tissue and serum pharmacodynamic markers and drug levels, these trials can provide more information than simple documentation of activity or lack of activity for a particular indication. Such information will be important: if the trials show activity, these data may guide further studies that will build on success (e.g., by defining subpopulations that benefit or by suggesting rational combinations). If the trials are negative, such companion studies will assist in interpretation: lack of activity might be reflected in technical issues that could be adjusted in follow-up studies (such as the use of a biguanide with a superior pharmacokinetic profile, if there is evidence for inadequate drug accumulation in tumors in a setting where a "direct" action was expected), or alternatively may provide evidence that the drug has no benefit even when conditions predicted to be necessary for activity are satisfied, justifying a decision to halt development for an indication.

Clues suggesting that metformin and/or related biguanides have antineoplastic activity are tantalizing, but clearly further multidisciplinary investigation is required to determine if these compounds actually will have a role to play in cancer prevention or treatment. Recent progress in defining critical roles of mitochondrial function in neoplasia, together with evidence for perturbation of mitochondrial function by biguanides, provide a rationale for research that extends beyond the original mechanistic hypotheses attributing biguanide effects to reduction in insulin levels, activation of AMPK, and inhibition of mTOR. Major areas of ongoing investigation are listed in Table 1.

Clinical trials in progress are examining the effects of metformin at conventional antidiabetic doses on various cancer endpoints to test the important hypothesis that this exposure level, which is known to be practical to administer on a long-term basis, has antineoplastic activity (89). Studies suggesting a variety of beneficial effects of metformin on aging (23), memory (112), and cardiovascular function (113) would argue that such trials should examine nononcologic health outcomes as well. However, many of the antineoplastic mechanisms of action of biguanides that operate in preclinical models may not be addressed in these trials, as drug accumulation in target tissues may not be sufficient. Methotrexate provides a classic precedent of a drug that is used as an antineoplastic at doses up to 100-fold higher than those used chronically for a separate indication (in this case, rheumatoid arthritis). Therefore, as a complementary approach, it will be important to proceed with the conventional phase I and II studies to assess the tolerability and efficacy of higher doses of various biguanides to determine clinical relevance of laboratory models showing activity at relatively high exposure levels. Such studies could, for example, involve relatively short-term use of phenformin at maximally tolerated doses, initially as a single agent, and then in rational combinations designed to maximize energetic stress in those cancers with defects in mechanisms that are required to survive this.

As often is the case in oncology, early clinical trials have been launched before relevant physiology and mechanisms are fully understood, and this creates both challenges and opportunities. To the extent possible, design of clinical trials should be guided by information concerning issues such as pharmacokinetics, rational drug combinations, and use of predictive biomarkers. Despite the logistic challenges, trials should incorporate companion translational research, bearing in mind that the cost of these studies is small relative to the overall cost of trial execution, yet the information gained can be strategically important. Although the private sector has had limited involvement in studies of metformin in view

Table 1. Biguanides: key areas of investigation in oncology

Topic	Questions	Comments
Clarifying pharmacoepidemiology	What will critical review of the retrospective data obtained from diabetic populations reveal?	Work is underway to interpret retrospective data in a manner that minimizes possible biases (114), and analysis of cancer incidence in cohorts where metformin use for diabetes treatment or prevention was randomized will be important.
Defining precise molecular target	What is the precise molecular basis of the action of biguanides in the mitochondria?	Recent evidence suggests that this may involve an interaction between biguanides and copper ions critical for oxidative phosphorylation (50), and other molecular mechanisms are under study.
Identifying the key mechanisms of action	How much of the antineoplastic activity of metformin is attributable to "endocrine"-type effects, such as the insulin-lowering effects proposed to slow tumor growth in hyperinsulinemic patients with insulin-sensitive cancers?	Such mechanisms imply long-term treatment for maximal benefit. Resistance mechanisms may eventually develop, as is the case for many long-term hormonal cancer therapies, but clinical benefits are nevertheless possible.
	How much of the antineoplastic activity of metformin is attributable to direct actions on target cells secondary to effects on energy metabolism, and of the many "direct" actions shown *in vitro,* which operate *in vivo* and do any operate clinically?	Do these direct mechanisms require long-term treatment, or are there contexts in which short-term higher dose biguanide exposure could have clinical use, perhaps in combination regimens?
Optimizing pharmacokinetics	Are there particular indications related to tissues known to have relatively high metformin levels following oral dosing, where pharmacokinetic considerations make metformin a particularly attractive biguanide to investigate in the context of the "direct" mechanisms of action? Examples: intestinal polyp prevention (Peutz–Jeghers syndrome, other polyposis syndromes, sporadic polyp prevention, and hepatoma risk reduction)	Relatively high levels are present in liver and the gastrointestinal tract following oral administration, suggesting possibilities in hepatoma risk reduction in high-risk patients or in colorectal cancer prevention (33, 108, 115).
	Are there anatomic sites where "direct" actions of metformin may be limited by pharmacokinetic considerations? If so, are observed activities in mouse models attributable to systemic effects? Possible examples are breast, prostate, and lung.	There are examples of models in which chemoprevention activity is seen, but drug accumulation in target tissues remains to be defined (e.g., ref. 116).
	Would cellular targeting strategies or the use of other biguanides overcome any pharmacokinetic limitations that might limit antineoplastic activity of metformin?	Phenformin is associated with higher risk of lactic acidosis than metformin, but nevertheless has a better safety profile than most antineoplastic agents in current use, and is more effective than metformin in preclinical models, probably because of its pharmacokinetic characteristics (75–77). There are libraries of many biguanides that could be screened for antineoplastic activity and/or used as lead compounds for optimization of pharmacokinetics.
	Are there species-specific factors that limit pharmacokinetic modeling in mice? In murine models, should research be confined to oral administration unless other routes are contemplated for novel administration methods clinically (e.g., short-term high-dose exposure following dosing of new intravenous formulations)?	There is uncertainty concerning the feasibility of administering biguanides by nonconventional routes to investigate therapeutic value of high-dose transient exposure.
Developing rational combinations	Does single-agent metformin deserve evaluation for indications in prevention? Are there any indications in cancer treatment for which single-agent use of metformin or another biguanide should be favored over rational combinations?	

(*continued*)

Table 1. Biguanides: key areas of investigation in oncology (*Continued*)

Topic	Questions	Comments
Developing rational combinations (*continued*)	With chemotherapy	Although there is uncertainty concerning mechanistic details, several studies (e.g., ref. 117) suggest a chemosensitizing effect of metformin.
	With glycolysis inhibitors	As there is evidence that increased glycolysis represents a resistance mechanism to the energetic stress induced by biguanides, there is a strong rationale to investigate such combinations. Although 2-deoxyglucose may not be practical for clinical use, cotargeting lactate dehydrogenase or enzymes required to process lactic acid are worthy of study (118–120).
	With steroid-targeting agents	Interactions with steroid synthesis deserve consideration in both breast and prostate cancer (73, 93).
	With PI3K inhibitors	PI3K inhibitors often lead to hyperglycemia and hyperinsulinemia, which may limit efficacy and increase toxicity. In this context, they are often combined with metformin in clinical trials, and may contribute to clinical benefit (5, 95).
	With salicylates	With the demonstration (121) that salicylate activates AMPK directly, it is of interest to consider the possibility of additive effects with biguanides particularly in the context of risk reduction; it is not rare for both drugs to be administered chronically.
	With VEGF inhibitors	AMPK activation, which can be a consequence of metformin exposure, can lead to increased VEGF expression and enhanced survival under certain conditions. There is preclinical evidence that inhibition of VEGF expression synergizes with metformin exposure to reduce cancer growth and oppose prosurvival consequences of AMPK activation (59–62).
Identifying predictive biomarkers	If biguanides have uses that vary between patients, can predictive biomarkers be identified?	There are precedents for drug development to require the use of predictive biomarkers. In the case of biguanides, candidates include tumor characteristics such as LKB1 status, the presence of transport molecules required for cellular accumulation of metformin in neoplastic tissue, and host characteristics such as BMI, IGFBP-1 level, or insulin level (64, 97, 122).
Prioritizing clinical trials	What are the most important contexts in which to carry out clinical trials of biguanides for cancer prevention or treatment?	Epidemiologic and laboratory studies to date do not clearly establish priority settings for trials, in terms of type of cancer, timepoint in natural history, combinations, or dose. Thus, ongoing trials are examining metformin for treatment of many different cancers, and in settings ranging from postsurgical adjuvant treatment to palliative treatment of metastatic disease. Few trials are examining rational combination therapies, and to date, all trials are exploring conventional antidiabetic doses. It remains to be determined, through conventional phase I and II programs, if strategies to expose tumors to the higher biguanide concentrations used in many preclinical models will be tolerated and/or useful in cancer treatment alone or in combinations, and if so, whether this involves mechanisms distinct from those that may operate with doses used in diabetes therapy.

of its status as a generic agent, this may change if novel biguanides are investigated.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Author's Contributions

Writing, review, and/or revision of the manuscript: M.N. Pollak

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