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Repurposing Old Drugs to Chemoprevention: The Case of Metformin

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Abstract

Multiple epidemiologic studies have documented an association between the anti-diabetic agent metformin and reduced cancer incidence and mortality. However, this effect has not been consistently demonstrated in animal models or more recent epidemiological studies. The purpose of this paper is to examine metformin's chemopreventive potential by reviewing relevant mechanisms of action, preclinical evidence of efficacy, updated epidemiologic evidence after correction for potential biases and confounders, and recently completed and ongoing clinical trials. Although repurposing drugs with well described mechanisms of action and safety profiles is an appealing strategy for cancer prevention, there is no substitute for well executed late phase clinical trials to define efficacy and populations that are most likely to benefit from an intervention.

Introduction

There is intense interest in the cancer prevention research community regarding the potential of metformin, the front-line oral treatment for type II diabetes (T2D), to prevent a wide variety of cancers. The increased risk of cancer in hyper-insulinemic states such as metabolic syndrome or T2D is well recognized ¹. Meta-analyses have estimated relative

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risks (RR) of 1.1-2.5 for cancer risk at various organ sites in patients with T2D ². These sites include liver, breast, bladder, endometrium, pancreas, colorectum, kidney, and non-Hodgkin lymphomas, among others. Metformin (1,1-dimethylbiguanide) is a cheap generic drug with a well understood safety profile and track record of tolerability that has been shown to decrease the progression from prediabetes to overt diabetes ³. Numerous epidemiologic studies and meta-analyses have documented an association between metformin use and reduced cancer incidence and mortality (fully discussed below), making metformin an ideal candidate for drug re-purposing. However, recent recognition of time-related biases as a major potential source of error in some epidemiologic studies calls for a re-evaluation of the literature linking metformin use to reduced cancer incidence ^{4, 5}. In this paper we examine the current state of understanding of the potential of metformin use to reduce cancer incidence and mortality, from putative mechanisms of action through the limited data on current and ongoing clinical trials.

Molecular mechanisms of metformin in cancer and diabetes

Despite its long history of clinical use in the diabetes setting, the precise mechanisms underlying the anti-hyperglycemic and anti-hyperinsulinemic effects, as well as the anti-cancer properties, of metformin are still incompletely understood. Two basic routes of metformin action have potential to contribute to its anti-neoplastic activity (Figure 1)^{6, 7}: (1) an indirect route involving “endocrine-type effects” related to its insulin-lowering activity which may slow tumor proliferation in hyperinsulinemic patients; and (2) direct actions in target cells resulting from its suppression of ATP production due to its inhibition of mitochondrial complex I ⁸. Both routes of metformin activity converge on the mTOR (mammalian target of rapamycin) pathway resulting in inhibition of its pro-proliferative activity. An important caveat to understanding the anti-proliferative effects of metformin is that they are context-dependent. As seen below, “context” here refers to the nutritional, energetic/oxidative state of the cell as well as to pharmacokinetic factors related to individual cell types ^{8, 9}.

Inhibition of the mTOR pathway

Key components of the mTOR pathway include LKB1 (serine-threonine liver kinase B1), AMPK (5'-adenosine monophosphate-activated protein kinase), TSC (tuberous sclerosis complex/tuberin), and mTOR (Figure 1), whereas upstream activators of mTOR include PI3K (phosphatidylinositol-3-kinase) and AKT (protein kinase B). In the setting of nutrient availability, growth factors such as insulin activate PI3K which, in turn, activates AKT which then phosphorylates and inactivates TSC2, counteracting the inhibitory effect that this complex has on mTOR. Thus, when amino acids and glucose are available, mTOR activation occurs. The mTOR molecule functions as part of a larger complex, mTORC1 (mTOR complex 1), which has several other components. These regulate mTOR's response to nutrients as well as to known mTOR inhibitors, such as rapamycin ¹⁰. Together the players in mTOR activation constitute a linear pathway that is responsive to activation by growth factors ¹⁰, promoting protein translation in part and leading to cell growth and proliferation, all in a setting of nutrient and energy availability (Figure 1).

Inhibition of mTOR activation takes place at several points along this linear pathway. The PTEN (phosphatase and tensin homolog) tumor suppressor protein restrains mTOR activity upstream by counteracting PI3K activity (Figure 1)¹⁰. Further downstream activation of AMPK serves a major role in mTOR inhibition¹¹. As a central cellular energy sensor and the key player in metformin-mediated direct inhibition of the mTOR pathway^{6, 11}, AMPK is activated by metformin and then phosphorylates and activates the mTOR inhibitor, TSC2. AMPK activation is dependent on phosphorylation of its catalytic subunit by its upstream activator, LKB1^{7, 12}, which is encoded by a tumor suppressor gene that is mutated in many nonsmall cell lung cancers and in the germline of cancer-prone patients with Peutz-Jeghers syndrome (Figure 1)¹²⁻¹⁴. Although LKB1 is required for this activation of AMPK by metformin, the LKB1 molecule itself is not the direct target of these drugs^{12, 15}. The LKB1/AMPK system functions as a sensor of ATP levels⁹. Furthermore, this LKB1/AMPK/TSC2 pathway is only one route by which metformin exerts its inhibitory effect on mTOR. In the absence of functional TSC2, metformin-activated AMPK can still inhibit mTOR/mTORC1 by directly phosphorylating the raptor component of the mTORC1 complex^{6, 16}. In addition, metformin has also been shown to inhibit mTORC1 in an AMPK-independent manner^{17, 18}.

In the indirect route of inhibiting neoplastic progression (referred to as route (1) above), pro-growth processes involving the insulin/insulin-like growth factor-1 (IGF-1) pathway are activated in a setting of nutrient availability⁶. In normal cells, following binding to its ligand, the IGF-1 receptor (IGF-1R) is phosphorylated, leading to a series of downstream phosphorylations that activate (a) the PI3K/Akt/mTOR and (b) the RAS/RAF/mitogen-activated protein kinase (MAPK) pathways (Figure 1). By activating both pathways, IGF-1/IGF-1R activation, stimulated by circulating insulin, contributes to increased cell growth and proliferation, ultimately pro-carcinogenic processes. Metformin acts on this pathway by suppressing IGF-1 signaling via inhibition of phosphorylation of IGF-1R. This decreases hepatic glucose output, increases muscle uptake of glucose, and reduces plasma insulin levels, with a concomitant antiproliferative effect that is “indirect” in relation to the ultimate cellular target, the cancer cell.

The second scenario, referred to as route (2) above, occurs when energy sources/glucose are not available and ATP levels are low, relying on AMPK, which is a keen sensor of cellular energy status¹¹, specifically the intracellular ratio of AMP to ATP. AMPK is directly activated when intracellular ATP concentrations decrease and intracellular AMP concentrations increase¹², leading to inhibition of the mTOR pathway. In cancer cells, the consequences of this process include decreased protein synthesis, which correlates with reduced energy expenditure, and a decrease in cell proliferation^{7, 19}. Metformin implements this second mechanism by acting as a mitochondrial poison, inhibiting complex I in the electron transport chain²⁰. This impairs production of mitochondrial adenosine-5'-triphosphate (ATP)^{19, 21} and creates a state of energy restriction, mimicking the naturally occurring state described for scenario (2). The resulting rise in the AMP:ATP ratio, with an increase in AMP, leads to increased binding of AMP to AMPK, which makes the AMPK a better substrate for LKB1¹⁵. Activation of LKB1-AMPK signaling results, leading to downregulation of phospho-AKT, mTOR, S6 kinase and 4EBP1 (4E binding protein 1) and the reprogramming of the metabolism of glucose and lipids. Of note, this effect of

metformin on the mitochondrion elicits AMPK activation¹³; metformin has not been shown to activate AMPK directly¹⁵. Metformin has been coined an “energy restriction mimetic agent” because of this ability to inhibit mitochondrial generation of ATP¹⁹.

In summary, the insulin-mediated pathway leads to synthesis of lipids, proteins and glycogen, in contrast to the AMPK pathway which inhibits these biosynthetic activities; yet, both mechanisms hone in on mTOR as a common target. The targeting of mTOR is funnelled through the TSC2/tuberin protein, which integrates the effects of the two pathways, insulin-generated growth and energy restriction (Figure 1)⁶.

Other anti-carcinogenic mechanisms of metformin

The pharmacokinetic and pharmacodynamic properties of metformin contribute to its anti-carcinogenic impact on specific cell types. Metformin can only directly affect tissues capable of taking up the drug. The organic cation transporter 1 (OCT1) protein is critical for uptake into hepatocytes^{7, 9, 22}. The 17-fold lower expression of OCT1 in lung tissue in a mouse lung cancer model has been invoked as a possible explanation for the inability of metformin to induce activation of AMPK in lung tissue in this model²³. Despite this, metformin has been shown to reduce the burden of lung cancer in mice, which it does by a mechanism that is independent of AMPK activation but instead involves the insulin pathway^{17, 23}.

The context dependence of metformin's anti-cancer activity is evident from studies suggesting selective targeting of cancer stem cells²⁴. Metformin has also been shown to inhibit cell cycle progression by preventing increases in cyclin D and E2F1 protein levels and phosphorylation of pRb in prostate cancer cells²⁵, resulting in G₀/G₁ arrest. Metformin induces cell death in cancer cells by both caspase-dependent, apoptotic and caspase-independent, nonapoptotic pathways^{23, 26}. In addition, metformin blocks migration and invasion of tumor cells, in part by inhibiting matrix metalloproteinase-9 activation²⁷. Metformin also exhibits anti-inflammatory and anti-oxidant properties, targeting mechanisms known to play roles in both obesity/diabetes and cancer²⁸. Both metformin and the mTOR inhibitor rapamycin have been shown to affect the immune system, enhancing memory T-cell development following resolution of acute infection (or cancer)²⁹. Finally, metformin has been shown to inhibit transcription of the aromatase gene, *CYP19A1*, in a manner that is selective for expression of this estrogen-synthesizing enzyme in the breast³⁰.

Animal carcinogenesis studies

The evidence for a cancer preventive effect for metformin, however, has not been consistently demonstrated *in vivo* in animal studies. Multiple studies examining the effect of metformin on breast tumors occurring spontaneously in outbred mouse strains or in transgenic Her2/neu mice or carcinogen-treated rodents have shown results ranging from no effect to minor, albeit statistically significant, decrease in tumor multiplicity and increase in latency when given early and at high doses^{19, 31-35}. Two of these studies showed an increase in female animal life span independent of effect on tumor incidence^{31, 33}, although in a study by Anisimov et al., the 4.4% increase in mean life span in females was counterbalanced by a 13.4% decrease in male animal mean life spans³⁴. A recent study

using both an ER-positive rat carcinogenesis model and Neu+/p53 knockout mice showed no effect of metformin administered at doses achievable in human beings³⁶. Of note, the tumor burden and proliferation index trended toward increases with metformin treatment, the latter reaching statistical significance.

Studies in colon cancer models showed modest to no effect on polyp number in azoxymethane-treated or Apc^{Min/+} mice, respectively, although large polyps were completely eliminated in the former model and 50% decreased in the latter^{37, 38}. Inhibition of mTOR phosphorylation was demonstrated in both models. Lung adenoma multiplicity and tumor volume in A/J mice treated with the tobacco carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) were mildly inhibited by oral metformin treatment, with little evidence for an effect on AMPK activation in the lung and minimal mTOR inhibition²³. Instead, circulating insulin and IGF-1 levels were reduced, suggesting an indirect effect on tumorigenesis. Intraperitoneal metformin treatment was more effective in reducing tumor burden and demonstrated enhanced mTOR inhibition, but still without effect on AMPK activation in the lung. On the other hand, studies using carcinogen-induced pancreas, oral, liver, skin, and prostate cancer rodent model systems showed much more profound suppression of tumor formation, which was associated with mTOR inhibition in some cases but independent of AMPK activation in other instances³⁹⁻⁴⁴. It should be noted that in the case of high-fat diet induced liver carcinogenesis, metformin treatment was effective during the early stages only, not by the time mice developed non-alcoholic fatty liver disease⁴³. Of note, the positive studies in pancreas, liver, and skin model systems were performed under high-fat conditions, which may have optimized the opportunities for metformin efficacy.

Epidemiologic studies

The epidemiologic literature regarding metformin use and cancer incidence and mortality, both overall and linked to specific organ sites, has exploded over the past decade. Multiple meta-analyses have now reported a decrease in cancer incidence of approximately 14-40%, along with a decrease in mortality in the same range (Table 1)⁴⁵⁻⁵⁰. Organ sites reported to be thus protected include breast, colon, liver, pancreas, prostate, endometrium, and lung, among others. However, these studies represent a multitude of trial designs, from cohort and case-control studies through randomized controlled trials. As the stringency of the study design increases, metformin's effect on cancer incidence appears to decrease. For example, Thakkar et al. found that while cancer incidence was decreased by 30% in cohort studies and 10% in case-control trials, there was no discernable effect in randomized controlled trials⁴⁹. We similarly performed a meta-analysis with particular attention to biases and confounders, and also found that the risk reduction for cancer incidence decreased from 31% (summary relative risk (SRR) =0.69; 95% Confidence Interval (CI), 0.52-0.90) for all studies to 29% (SRR=0.71; 95%CI, 0.47-1.07) for prospective studies to 5% (SRR=0.95; 95%CI, 0.69-1.30) for randomized clinical trials⁵.

It has been pointed out recently that the existing metformin literature is subject to important sources of time-related bias, which potentially exaggerate cancer-reducing effects⁴. Time-related bias is most often a form of differential misclassification bias that can generally be avoided by appropriate accounting of follow-up time and exposure status in the design and

analysis of such studies. The different types of time-related biases include immortal time bias (unexposed time is misclassified as drug-exposed time), time-window bias (differential exposure opportunity time windows between exposed and unexposed subjects), and time-lag bias (comparison of treatment given during different stages of the disease). Immortal time bias refers to a period of cohort follow-up time during which a cancer event (that determines end of follow-up) cannot occur. Immortal time bias, for example, can arise when the period between cohort entry and date of first exposure to metformin, during which cancer has not occurred, is either misclassified or simply excluded and not accounted for in the analysis. This is frequently found in studies that compare ‘users’ against ‘non-users’. In cohort studies where a first-line therapy with metformin is compared with second- or third-line therapies, patients are unlikely to be at the same stage of diabetes, which can induce confounding of the association with an outcome (e.g., cancer incidence) by disease duration. An outcome related to the first-line therapy may also be attributed to the second-line therapy if it occurs after a long period of exposure. Such a situation requires matching on disease duration and consideration of latency time windows in the analysis.

In our meta-analysis, we found that adjustment for time-related biases resulted in a diminution of the protective effect on cancer incidence⁵. The risk reduction in cancer incidence at all sites decreased from 31% for all studies (19 studies, SRR=0.69; 95% CI, 0.52-0.90) to 10% for studies adjusted for time-related biases (8 studies, SRR=0.90; 95% CI, 0.89-0.91), albeit still statistically significant. With regard to specific organ sites where at least 3 studies could be appropriately evaluated, metformin use was significantly associated with decreased breast cancer incidence after adjustment for time-related biases (SRR= 0.94; 95%CI, 0.90-0.99) compared with a 12% non-significant decreased incidence in all studies (SRR=0.88; 95%CI, 0.75-1.03), but the magnitude of the effect was rather small. For colon cancer, a similarly small 8% decrease in incidence was noted (SRR=0.92; 95%CI, 0.85-0.98) that compared with a 20% marginally significant decrease when all studies were considered (SRR=0.80; 95%CI, 0.64-1.00). For lung cancer, examination of all studies showed an 18% decrease in cancer incidence (SRR=0.82; 95%CI, 0.67- 0.99). Adjustment for time-related biases decreased this protective effect to a statistically significant 12%, but adjustment for smoking, which is by far the most relevant lung cancer risk factor, resulted in no association between metformin use and cancer incidence. Similarly, the strong protective effect of metformin use against liver cancer (9 studies, SRR=0.47; 95%CI, 0.28-0.79) became non-significant when only the 3 non-time biased studies were examined (SRR=0.65; 95%CI, 0.39-1.08). It should be emphasized, however, that the total number of studies and patients was rather small for the liver cancer associations and thus these results should be interpreted with caution. Finally, for prostate and pancreatic cancers, metformin use was not associated with a statistically significant protective effect.

Another potential source of confounding that is often not adequately explored in the existing literature is the effect of obesity and its surrogate, body mass index (BMI). Obesity is intimately linked to increased risk of multiple cancer types². Potential mechanisms include both direct and indirect effects of obesity on insulin, IGF-1, sex hormones, adipokines, and inflammation, many of which are directly impacted by metformin. Metformin, unlike several other anti-diabetic agents, is associated with weight loss. Furthermore, a recent clinical trial showed that metformin affected breast cancer proliferation differentially

according to insulin resistance status and BMI, with a trend toward inhibiting proliferation only in women with insulin resistance or high BMI (see below)⁵¹. Therefore, we examined BMI as a potential confounder in our meta-analysis and found that adjustment for BMI decreased the protective effect of metformin for all-cancer incidence from 31% to 18%, which was still statistically significant⁵. For breast cancer, adjustment for BMI revealed a borderline association of metformin use with cancer incidence (SRR=0.82; 95%CI, 0.67-1.00). For colon and prostate cancer, there was no statistically significant association between metformin use and cancer incidence. Other organ sites could not be assessed due to a small number of reported studies. It should be noted, however, that BMI is dynamic and therefore adjusting for a single BMI value might be inadequate to account for confounding by BMI dynamics over time. Furthermore, we were unable to assess the effect of BMI and time-related biases simultaneously due to inadequate numbers of studies that controlled for both factors.

The effect of metformin on cancer mortality is informed by a much smaller literature and thus we were unable to determine the effect on isolated cancer sites⁵. With regard to all-cancer mortality, metformin use was associated with a 34% decrease (7 studies, SRR=0.66; 95%CI, 0.54-0.81) that remained significant when the analysis was limited to only prospective trials (4 studies, SRR=0.48; 95%CI, 0.23-0.97). Adjustment for BMI maintained the magnitude of the effect (5 studies, SRR=0.60; 95%CI, 0.45-0.80), although adjustment for time-related biases, limited to only 3 studies, resulted in loss of statistical significance (SRR=0.45; 95%CI, 0.16-1.26). Different mechanisms may be responsible for metformin's effect on cancer mortality compared with cancer incidence. Several retrospective analyses have suggested that diabetics treated with metformin during chemotherapy have longer survival than individuals treated with other anti-diabetic agents^{52, 53}. A previous mouse xenograft study showed that metformin targets breast cancer stem cells and synergizes with doxorubicin to prevent relapse⁵⁴. Increasing the effectiveness of chemotherapy could result in improved survival.

Taken together, these results point out the limitations of the current literature regarding the association between metformin use and cancer incidence and mortality. While all the studies, including our own meta-analysis, suggest that metformin use is associated with a reduced risk of cancer and death from cancer, the effect may be far smaller than previously believed.

Review of Clinical Trials

Although the literature reviewed above suggests a potential role for metformin in cancer prevention, well-crafted clinical trials addressing specific research questions in uniform cohorts will be needed to eventually determine if metformin is, indeed, effective in reducing cancer incidence. Here we review the status of completed and ongoing clinical trials that address the potential efficacy of metformin to prevent cancer in a variety of clinical settings. Eleven early phase studies of metformin in individuals at risk for or with cancer have been published (Table 2). Fifty-one additional studies are registered in clinical trial registries. Fifteen trials are being conducted in various high cancer risk populations (Table 3).

Seventeen studies are being conducted in the presurgical setting (Table 4) and 20 studies are registered in the adjuvant setting (Table 5).

Completed Clinical Trials

Two studies examined various doses of metformin given for 3-6 months to women after therapy for breast cancer (Table 2). The first published study examined women with breast cancer who completed adjuvant therapy and whose plasma levels of insulin were at least 45 pmol/L (Table 2) ⁵⁵, based on positive results from a prior cohort study ⁵⁶. Metformin decreased circulating insulin levels by 22.4% (p=0.0024), although 25% of participants dropped out prematurely. A second study in women with breast cancer who had completed adjuvant therapy but had elevated testosterone compared two different doses of metformin, demonstrating that the higher dose significantly reduced serum testosterone levels and free androgen index compared to the lower dose ⁵⁷.

Five trials examined the short-term effects (1-4 weeks) of various doses of metformin on cell proliferation (Ki-67) in women awaiting surgery for breast cancer (pre-surgical trials). The first trial found a 3.4% reduction in Ki-67 (p=0.027) in the metformin arm, but 29% of the patients on metformin withdrew due to gastrointestinal side effects ⁵⁸. In a second larger double-blind, placebo-controlled study, the metformin effect on Ki-67 change relative to placebo was not statistically significant, with a mean proportional increase of 4.0% ⁵¹. However, women with a HOMA index (Homeostasis Model Assessment, used to quantify insulin resistance) >2.8 had a non-significant decrease of 10.5% while women with a HOMA index <2.8 had a non-significant increase of 11.1%. The interaction between HOMA index and metformin on Ki-67 was statistically significant (p=0.045); a similar trend was seen with BMI, although it was not statistically significant. With dose escalation of metformin, patient drop-out was not problematic ⁵¹. A third single arm trial reported a 3% decrease in Ki-67 (p=0.016) after a median of 18 days of treatment ⁵⁹ and a recently completed study showed no reduction in Ki-67 ⁶⁰.

Endometrial cancer prevention is of particular interest because of its relationship with obesity and the use of metformin for treatment of polycystic ovary syndrome, a condition associated with increased endometrial cancer risk. Two studies have been reported in women with early stage endometrial cancer. With a dose of 850mg QD, a single arm study of stage 1-2 endometrioid endometrial cancer showed an 11.75% reduction in Ki-67 ⁶¹. In a study that increased the dosage from 750mg QD to 1500mg or 2250 mg, 4 weeks of metformin resulted in a 44.2% decrease in Ki-67 ⁶².

Three additional studies in other organ systems (Barrett's esophagus, colon, and prostate) have been reported. A study in Barrett's esophagus showed no significant change in phosphor-serine 6 kinase (pS6K), cell proliferation, or apoptosis ⁶³. Another study examined change in the number of aberrant crypt foci (ACF, a putative precursor of colon cancer) in individuals with pre-existing ACF, randomizing participants to a very low dose of metformin (250 mg/day) or no treatment for one month ⁶⁴. A significant decrease in ACF from 8.78 ± 6.45 to 5.11 ± 4.99 (p=0.007) was observed in the metformin arm but not in the control group. Additionally, proliferation was significantly decreased in the metformin group (p<0.001). Finally, men with prostate cancer were treated with metformin for 4-12

weeks prior to surgery. The primary endpoint, change in Ki-67, was statistically significant, with an absolute decrease of 1.44% and a relative decrease of 29.5%⁶⁵.

Ongoing Clinical Trials

The ongoing studies (Tables 3-5) examine similar endpoints, including pharmacodynamic markers such as tumor/tissue levels of pS6K or other measures of AMPK-activating kinase (AMPK) signalling. Many of the studies examine surrogate endpoints for cancer such as atypia for breast cancer or change in prostate specific antigen (PSA) for prostate cancer. Over 5,000 participants are expected to enroll in these trials examining the effect of metformin on tumorigenesis in multiple organ systems. Not all trials are discussed in detail in the text, which discusses the studies by organ site, but a complete list is available in the tables based on disease setting (prevention, presurgical, or adjuvant).

Thirteen additional studies are examining the effect of metformin in the breast. These include a study in women scheduled for a reduction mammoplasty that compares changes in LKB1 and AMPK signaling in women treated with metformin 500 mg twice a day (dose escalated) versus no treatment (Table 3, ACTRN12610000219088)⁶⁶ and two larger studies one in overweight and obese premenopausal women with high breast density (Table 3, NCT01793948) and one in high-risk premenopausal women with cytologic atypia (Table 3, NCT01905046), four additional single-arm pre-surgical breast studies examining changes in Ki-67 and AMPK signaling following metformin 1000-1700 mg/ day for 2-3 weeks (Table 4, NCT00930579, NCT01266486, NCT01589367, NCT01929811)⁶⁷⁻⁷⁰ and two presurgical studies that were terminated due to poor accrual (Table 4, NCT01302002 and NCT00984490), and four studies in the adjuvant setting. The largest of these is the NCIC MA.32 trial, a phase III adjuvant breast cancer trial randomizing 3649 women within 12 months of diagnosis to metformin 850 mg twice a day (850 mg/day during weeks 1-4) versus placebo for 5 years (Table 5, NCT01101438)⁷¹. The primary endpoint is invasive disease free survival. The three other adjuvant studies include a trial examining the effects of metformin 500 mg/day, 500 mg twice a day, or placebo for 6 months on weight loss in breast cancer survivors with a BMI of ≥ 23 who have completed chemotherapy and radiation (Table 5, NCT00909506)⁷², the “Reach for Health Study” that is randomizing postmenopausal breast cancer survivors with a BMI of ≥ 25 to metformin, exercise, the combination, or standard dietary guidelines for 6 months to determine the effects on biological markers associated with breast cancer survival (Table 5, NCT01302379)⁷³, and a pilot study examining the effect of metformin 850 mg twice daily in combination with omega-3 fatty acids in women with a history of stage 0-III breast cancer 6 months since completion of all therapy for 1 year to determine feasibility (Table 5, NCT02278965).

Five clinical trials are examining metformin in the colon. Two trials are studying individuals with a previous history of colorectal adenomas. One study treats subjects with BMI ≥ 30 with metformin dose escalated from 500 -2000 mg per day for 12 weeks and has a primary endpoint of change in pS6K-serine 235 (Table 3, NCT01312467)⁷⁴. A placebo-controlled, 2 dose trial studying individuals with familial adenomatous polyposis for 7 months examining mean change in number of polyps (Table 3, NCT01725490). One study is recruiting patients with newly diagnosed colon cancer who are scheduled to undergo surgery while 2 were

terminated (Table 4, NCT01440127, NCT01816659). The active study is examining the effect of 2-3 weeks of metformin 850 mg twice a day versus placebo on proliferation and apoptosis in colon tumors and adjacent normal tissue (Table 4, NCT01632020) ⁷⁵. The effect of combination of metformin plus exercise is also under study in a trial of colorectal cancer survivors (with the addition of breast cancer survivors as well due to slow accrual) who are randomized to metformin alone dose escalated to 850 mg twice a day, exercise, metformin plus exercise, or education for 1 year with a primary endpoint of change in fasting insulin at 6 months (Table 5, NCT01340300) ⁷⁶.

Eight additional studies are being conducted in women with endometrial hyperplasia or early stage endometrial cancer. A pilot study is examining whether metformin dose escalated from 750 to 1500mg per day for 4 years in combination with 400 mg medroxyprogesterone acetate (MPA) and aspirin 100 mg/day for the first 24 weeks can affect the recurrence free interval in women diagnosed with stage 1A endometrial adenocarcinoma or atypical endometrial hyperplasia (Table 5, JPRN-UMIN000002210) ⁷⁷. Three additional studies are examining the effect of biomarkers in women with newly diagnosed early stage endometrial cancer (Table 4, NCT01205672, NCT01877564, NCT02042495). Two studies are examining the combination of metformin with Mirena, a levonorgestrel-releasing intrauterine system, in women with endometrial hyperplasia (Table 3, NCT02035787, NCT01686126). One study is examining the change in endometrial proliferation comparing exercise, exercise plus metformin, metformin, and placebo in women with a BMI greater than or equal to 30 (Table 3, NCT01697566) and an additional study is examining the response rate of endometrial hyperplasia in a phase 0 pilot study (Table 3, NCT01685762).

Ten prostate cancer studies are ongoing. A pre-prostatectomy study is examining Ki-67 and drug concentrations in the prostate tissue after 4-12 weeks of metformin dose escalated to 1500 mg per day (Table 4, NCT01433913) ⁷⁸. Four studies are examining the effect of metformin on PSA response in asymptomatic men with advanced or recurrent prostate cancer (Table 5, NCT01215032, NCT01243385, NCT01620593, NCT02176161). One of these studies is examining PSA doubling time in men after completion of initial therapy (Table 5, NCT02176161). A study comparing metformin to the combination of metformin and simvastatin on PSA doubling time was terminated due to poor enrollment (Table 5, NCT001561482). A Phase III randomized, placebo-controlled study in men with biopsy-proven, low-risk localized prostate cancer who are undergoing active surveillance is examining time to progression (Table 3, NCT01864096).

Two other Phase III studies are also ongoing. A phase III study examining the effect of metformin on progression of viral Hepatitis C cirrhosis to liver cancer is ongoing in France (Table 3, NCT02319200). In the US, participants in the Diabetes Prevention Program, a phase III study that started in 1996 to examine the influence of metformin and intensive lifestyle intervention on progression of pre-diabetes to diabetes, has retrospectively collected cancer outcomes and will continue to collect cancer outcomes as part of an extension to the original clinical trial (Table 3, NCT00038727).

Conclusions

The emerging data from preclinical, epidemiologic, and clinical studies suggest that there is a signal for cancer preventive potential with metformin use. There is biologic plausibility for a cancer preventive effect given the multiple ways that metformin can interfere with cancer promoting signalling pathways. However, both animal and epidemiologic studies have shown somewhat mixed effects. Further, the epidemiologic literature reviewed above relates only to individuals with diabetes and the effect is of lesser magnitude than previously reported once the appropriate adjustments to avoid bias and confounding are made. It remains to be determined whether a similar protective effect can be demonstrated in non-diabetic individuals. Multiple ongoing clinical trials are addressing which patient cohorts at risk for specific diseases are most likely to benefit from metformin. Although the long history and clinical experience with metformin make it a very attractive candidate for drug repurposing, general recommendations about its use, particularly in non-diabetic populations, need to await the results of the ongoing studies.

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Figure 1.

Two basic routes of metformin action have potential to contribute to its anti-neoplastic activity. (1) The indirect route involves “endocrine-type effects” related to its insulin-lowering activity (left arrow from “Metformin” box); this action may slow tumor proliferation in hyperinsulinemic patients. (2) Direct actions in target cells result from metformin's suppression of ATP production due to its inhibition of mitochondrial complex I (right arrow from “Metformin” box). Both routes of metformin activity converge on the mTOR (mammalian target of rapamycin) pathway resulting in inhibition of mTOR's pro-proliferative activity.

Table 1

Meta-analyses of Trials Examining Effect of Metformin on Cancer Incidence and Mortality

Study	Cancer Incidence Summary Relative Risk (95%CI)	Cancer Mortality Relative Risk (95%CI)
DeCensi et al., 2010 ⁴⁵	0.68 (95%CI, 0.52-0.88)	0.70 (95%CI, 0.51-0.96)
Noto et al., 2012 ⁴⁷	0.67 (95%CI, 0.53-0.85)	0.66 (95%CI, 0.49-0.88)
Soranna et al., 2012 ⁴⁸	0.61 (95%CI, 0.54-0.70)	NR
Thakkar et al., 2013 ⁴⁹	0.70 (95%CI, 0.67-0.73) Coh 0.90 (95%CI, 0.84-0.98) CC 1.01 (95%CI, 0.81-1.26) RCT	NR
Franciosi et al., 2013 ⁴⁶	0.73 (95%CI, 0.61-0.88) 0.98 (95%CI, 0.81-1.19) RCT	0.65 (95%CI, 0.53-0.80)
Gandini et al., 2014 ⁵	0.69 (95%CI, 0.52-0.90) 0.95 (95%CI, 0.69-1.30) RCT	0.66 (95%CI, 0.54-0.81)
Wu et al., 2015 ⁵⁰	0.86 (95%CI, 0.83-0.90) 0.88 (95%CI, 0.83-0.92) Coh 0.71 (95%CI, 0.63-0.80) CC 1.05 (95%CI, 0.94-1.18) RCT	0.70 (95%CI, 0.53-0.94) 0.66 (95%CI, 0.49-0.89) Coh 0.91 (95%CI, 0.37-2.23) RCT

95%CI=95% Confidence Interval; NR=not reported; Coh=cohort study; CC=case-control study; RCT=randomized control trial

Table 2

Published Metformin Clinical Trials

Organ Site; Phase; ClinicalTrials.gov, EudraCT or ICTRP Number	Study Design	Study Population	Planned Accrual/Evaluable	Primary Outcome
Barrett's Esophagus Phase II NCT01447927 ⁶³	500-2000mg qd over time metformin vs. placebo for 12 weeks	Barrett's esophagus 2cm in length, daily PPI for 4wks, excluding high-grade dysplasia	74	No significant change in pS6K1
Breast Phase II ⁵⁵	500mg tid for 6 months	Women with IBC who completed therapy with fasting insulin of 45 pmol/L and glucose < 7.0 mmol/L	40/22	Change in insulin levels 22.4% decrease (p=0.024)
Breast Phase I NCT00897884 ⁵⁹	500mg bid for 2-3 weeks	Women <70 Presurgical- IBC T1-4	48/39	2.97% decrease in Ki-67 (p=0.016)
Breast Phase II 2008-004912-10 ⁵¹	850mg/d for 3 days followed by 850mg bid day 4-28 Vs. Placebo for 4 weeks prior to surgery	Women Presurgical-Stage I- III IBC patient not suitable for neoadjuvant therapy	200/196	No overall change in Ki-67 10.5% decrease in Ki-67 if HOMA >2.8 (p for interaction =0.045)
Breast Phase II 2007-000306-70 ⁵⁸	500mg/d for 1 week followed by 1000mg/d for 1 week Vs. Placebo	Stage 1-2 IBC, >1cm, no history of diabetes	47/39	3.4% decrease in Ki-67 (p=0.02)
Breast Phase 0 NCT01980823 ⁶⁰	500mg am and 1000mg pm metformin with 80mg atorvastatin for at least 2 weeks prior to surgery	Histologically confirmed DCIS or IBC who undergo CNB followed by surgery	40	No reduction in Ki-67
Breast Phase II 2006-006236-22 ⁵⁷	1000 vs. 1500 mg/d for 3 months	Postmenopausal with history of IBC and 6 mos post- surgery, on tamoxifen for at least 6 mos and plan to continue, or at least 6 mos post chemo	125/96	1500mg/d decreased testosterone by 23% (p<0.01)
Colon Phase II ⁶⁴	250 mg qd vs. control for 4 weeks	Presence of ACFs, no concurrent use of NSAIDs	26/23	Number of ACFs decreased from 8.78 ± 6.45 to 5.11 ± 4.99 P = 0.007 for metformin arm
Endometrial Phase 0 NCT01911247 ⁶¹	850mg qd for 1-4 weeks	BMI 30 or greater with confirmed type 1 endometrial cancer	28/20	11.75% reduction in Ki-67 (p=0.008)
Endometrial Phase I JPRN- UMIN000004852 ⁶²	750mg/d increased to 2250mg/d if no side effects prior to surgery	Women age 20-70 with grade 1-2 histologically confirmed EA without myometrium invasion	40/31	44.2% decrease in Ki-67 (p<0.001)
Prostate Phase I NCT00881725 ⁶⁵	500mg tid for 4-12 weeks prior to surgery	Confirmed PC scheduled for prostatectomy	40/22	29.5% decrease in Ki-67 (p=0.006)

IBC-invasive breast cancer, DCIS- ductal carcinoma in situ, qd- one a day, bid- twice a day, tid- three times a day, EIO- European Institute of Oncology, Tam-Tamoxifen, T- Testosterone, ACFs- aberrant crypt foci, NSAIDs- non-steroidal anti-inflammatory drugs, BMI- body mass index, PC- prostate cancer, PPI-proton pump inhibitor, HOMA- Homeostasis Model Assessment, CNB- core needle biopsy, EA- Endometrial adenocarcinoma

Table 3

Metformin Cancer Prevention Trials – High Risk Populations (N=15)

Organ Site; Phase; ClinicalTrials.gov , EudraCT or ICTRP Number, Status	Study Design	Study Population	Target Accrual	Primary Endpoint
All Phase III NCT00038727, O	1850mg/d metformin, intensive lifestyle vs. placebo	Pre-diabetes	3234	Development of diabetes Secondary: Development of cancer
Barrett's Esophagus Phase I NCT01312467, C	500-2000mg/d over time metformin for 12 weeks	Barrett's esophagus 2cm in length, daily PPI for 4weeks, excluding high-grade dysplasia	50	% Change in pS6K
Barrett's Esophagus Phase 0 NCT01465113, R	50,000IU weekly Vitamin D3 with 1000mg bid metformin (dose escalated over 4 weeks) vs. vitamin D alone for 12 weeks	Barrett's esophagus 2cm in length, daily PPI for 4weeks, excluding high-grade dysplasia	66	15-prostaglandin expression
Breast Phase I ACTRN12610000219088	500mg/d for 1 week followed by 1000mg/d for 4 weeks prior to reduction mammoplasty	Women age 40-60	60	AMPK signaling and aromatase expression
Breast Phase II NCT02028221, R	850mg for 1 month followed by 850mg bid for an additional 11 months vs. placebo	Premenopausal women age 30-45 with BMI of 25 or greater and have metabolic syndrome	150	Change in breast density from baseline at 6 and 12 months
Breast Phase II NCT01793948, R	850mg qd for 30 days and bid for 11 months vs. placebo	Postmenopausal and high risk for breast cancer with BMI 25 or greater and high breast density	24	Changes in phosphorylated proteins by RPPA
Breast Phase III NCT01905046, R	850mg qd for 4 weeks followed by 850mg bid vs. placebo for 24 months. Placebo group may cross over to metformin for months 13-24.	Premenopausal, prior AH, LCIS or DCIS, >1.66% Gail or known BRCA carrier, MD >25% and cytological atypia	400	Regression of atypia at 12 and 24 mos
Colon Phase II NCT01312467, C	500mg d1-7, 1000mg d8-14; 1500mg d15-21, 2000mg d22-end of study endoscopy	Age 35-80; history of adenoma in previous 3 yrs; BMI 30	50	Reduction in pS6K-S235
Colon Phase II NCT01725490, R	500mg qd vs 500mg tid vs. placebo 2:2:1 for 7 mos	FAP, age 20-65 excluding patients who use NSAIDs	100	Mean percentage change in the number of polyps
Endometrial Phase 0 NCT01685762, R	850mg qd for 4 weeks then 850mg BID for 8 weeks	EH without atypia	15	Response rate
Endometrial Phase II NCT02035787, R	850mg bid with Levonorgestrel-releasing IUD for 12 months	Non-surgical candidate for histologically confirmed CAH or grade 1 endometrial cancer	30	Response rate – compared to base rate of 50%
Endometrial Phase II NCT01686126, R	Levonorgestrel-releasing IUD vs Levonorgestrel-releasing IUD plus metformin 500mg bid, vs. Mirena plus Weight Watchers for 6 months	Non-surgical candidate for histologically confirmed CAH or grade 1 endometrial cancer	165	pCR
Endometrial Phase II NCT01697566, R	Exercise, exercise + 850mg bid, 850mg bid, vs placebo for 4 months (weekly dose escalation)	Women BMI 30	100	Change in Ki-67

Organ Site; Phase; ClinicalTrials.gov , EudraCT or ICTRP Number, Status	Study Design	Study Population	Target Accrual	Primary Endpoint
Liver Phase III NCT02319200, NO	1000mg bid metformin vs. placebo for 36 months	Patients with viral Hepatitis C cirrhosis	444	Rate of HCC occurrence and liver related death or transplantation
Prostate Phase III NCT01864096, R	850mg once daily for 1 month followed by 850mg bid for 35 months	Biopsy-proven, low risk localized prostate cancer, undergoing active surveillance	408	Time to progression

R- recruiting, NO- not open yet, C- completed, O- ongoing, not recruiting, qd- one a day, bid- twice a day, tid- three times a day, BMI- body mass index, LCIS- lobular carcinoma in situ, DCIS- ductal carcinoma in situ, PPI- proton pump inhibitor, RPPA, reverse phase protein array, MD- mammographic density, NSAIDs- non-steroidal anti-inflammatory drugs, FAP – familial adenomatous polyposis, EH- endometrial hyperplasia, CAH- complex atypical hyperplasia, pCR- pathologic complete response, IUD- intrauterine device, HCC- hepatocellular carcinoma, pS6K-S235- phospho-serine 6 kinase -serine 235

Table 4**Metformin Presurgical Trials (N=16)**

Organ Site; Phase; ClinicalTrials.gov, EudraCT or ICTRP Number, Status	Study Design	Study Population	Target Accrual	Primary Endpoint
Breast Pilot NCT00930579, O	500mg in morning and 1000 mg in the evening for 2 weeks	Women Presurgical- DCIS or operable IBC with BMI>25	35	AMPK/mTOR signaling
Breast Phase 0 NCT01302002, T	500mg bid for 3 weeks	Women Presurgical- IBC T1 or T2	30	Ki67, TUNEL, pAKT
Breast Phase I NCT00984490, T	850mg bid for 7-21 days	Women Presurgical- IBC Stage I-IIIa	5	% Change in Ki-67
Breast Phase II NCT01266486, C	1500mg/d for 14-21 days prior to neoadjuvant chemotherapy	Women with IBC with no prior treatment	41	Change in pS6K, p4E-BP-1 and pAMPK
Breast Phase II NCT01589367, R	Letrozole 2.5mg with metformin 500mg bid for 1 week, 1000mg morning, 500mg evening for 1 week then 1000mg bid for 22 weeks or placebo	ER-positive breast cancer with no evidence of metastasis	208	CRR
Breast Phase II NCT01929811, R	500mg tid (500mg qd in cycle 1) with TEC vs. TEC alone until disease progression	Stage IIB or III with BMI 25 or greater	200	pCR
Bladder Phase II NCT02360618, R	850mg qd metformin and 20mg simvastatin for 12 weeks prior to surgery	Biopsy proven invasive bladder cancer	44	Change in Ki-67
Colon Phase II NCT01632020, O	1700mg bid vs. placebo 10-21 days prior to surgery	Newly diagnosed colon or rectal adenocarcinoma	40	Change in proliferation and apoptosis of tumor and surrounding normal
Colon Phase I NCT01440127, T	500mg bid 7-14 days prior to surgery	Colorectal cancer diagnosis undergoing surgery or biopsy	9	Expression of CD133 in tumors metformin vs. control
Colon Phase I NCT01816659, T	500mg qd for 1 week with escalation to 200mg qd for up to 30 days prior to surgery	Colorectal cancer or FAP with planned surgery	3	Ki-67
Endometrial Phase I NCT01205672, O	850mg/d for 7-30 days	Women age 18-80 with histologically confirmed endometrial adenocarcinoma	30	Change in S6K expression
Endometrial Phase II NCT01877564, R	Metformin vs. no treatment prior to surgery	Histological confirmed diagnosis of grade I or II endometrial adenocarcinoma with BMI 30 or greater	40	Ki-67
Endometrial Phase II NCT02042495, NO	500mg tid with possible dose reduction to 500mg bid for 4-6 wks	Confirmed diagnosis of endometrial cancer with planned surgical staging	80	pS6K
Head and Neck Phase 0 NCT02402348, R	850mg/d for 3 days, 850mg bid day 4 until surgery	Newly diagnosed HNSCC Stage II-IV	30	% Tumor cell death
Head and Neck Phase 0 NCT02083692, R	500mg qd days 1-3, 500mg bid days 4-6, 1000mg bid starting day 6 for 9-28 days	Biopsy proven head and neck cancer with planned definitive resection	40	Change in TOMM20 and MCT4
Prostate Phase I NCT01433913, C	500mg/d d1-7, 1000mg/d d8-14, 1500mg/d d22-surgery; total study length 4-12 weeks	Men scheduled for prostatectomy	21	Change in Ki-67

R- recruiting, NO- not open yet, C- completed, O- ongoing, not recruiting, T- terminated, qd- one a day, bid- twice a day, tid- three times a day, TEC- docetaxel, epirubicin and cyclophosphamide, BMI- body mass index, IBC-invasive breast cancer, DCIS- ductal carcinoma in situ, FAP- familial adenomatous polyposis, pCR- pathologic complete response, ER- estrogen receptor, AMPK- AMP-activated protein kinase, mTOR- mammalian target of rapamycin, TUNEL- terminal deoxynucleotidyl transferase dUTP nick end labeling (apoptosis), pS6K- phospho-serine 6 kinase, p4E-BP- phosphor-eukaryotic translation initiation factor 4E binding protein, CD133- cancer stem cell marker, CRR- complete response rate, HNSCC- head and neck squamous cell carcinoma, TOMM20- translocase of outer mitochondrial membrane 20, MCT4- monocarboxylate transporter 4

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Table 5

Metformin Adjuvant Trials (N=20)

Organ Site; Phase; ClinicalTrials.gov , EudraCT or ICTRP Number, Status	Study Design	Study Population	Target Accrual	Primary Endpoint
All Phase I NCT01981525, R	500mg qd for 1 week, 500mg bid for 1 wk, 500mg tid for 1 week and 1000mg bid for 1 wk if tolerated continued treatment for 14 weeks	<i>TP53</i> germline mutation at least 18 yrs old. If prior cancer at least 6 months from surgery and 1 year from chemotherapy	36	Tolerability and effect on IGF-1, insulin, IGFBP-3
Breast Phase II NCT01302379, R	Exercise, exercise + metformin 500mg at dinner (1 weeks) 1000mg at dinner (2-4 weeks) 500mg in morning and 1000mg at dinner, metformin + standard dietary guidelines, Vs. dietary guidelines for 6 months	Postmenopausal IBC survivor Stage 1-3A within past 4 yrs, completed chemotherapy, BMI 25	340	Biological markers associated with breast cancer survival
Breast Phase II NCT00909506, U	500mg/d vs 1000mg/d vs. Placebo for 6 months	BC survivor with BMI 23 6-24 months since surgery and 4 weeks since chemotherapy or radiation	105	Weight loss
Breast Phase III (MA. 32) NCT01101438, O	850mg bid vs. Placebo for 5 yrs (850mg qd for first 4 weeks)	IBC Survivor 4 weeks post therapy	3649	Invasive disease-free survival
Breast Phase III NCT01666171, U	Ancillary study to MA.32	ER-negative IBC with 25% or greater MD with contralateral breast available for analysis	458	Change in % mammographic density
Breast Phase III NCT01286233, O	Ancillary Study to MA.32	DNA available	394	QoL
Breast Pilot NCT02278965, R	850mg bid metformin and 1120mg bid omega-3 fatty acids	History of Stage 0-III breast cancer, 6 months since completion of chemotherapy, biologic therapy, and tamoxifen	20	Number of participants completing 1 year intervention
Colon/ Breast Phase II NCT01340300, R	Exercise, exercise + metformin 850mg qd for 2weeks then 850mg bid metformin, vs. educational information for 1 year	Stage 1-3 breast or colorectal cancer survivor completed all adjuvant therapy within 2-24 months prior to enrollment	200	Change in fasting insulin levels at 6 months
Endometrial Pilot JPRN- UMIN000002210, C	400mg/d MPA, 100mg/d aspirin for 24 weeks with 750mg/d metformin continuously for 4 years (increased to 1500mg/d if no side effects)	Histologically well differentiated EA at presumed stage IA and atypical endometrial hyperplasia	30	Recurrence free interval
Lung Phase II NCT01717482, R	850mg qd for 4 weeks followed by 850mg BID for a total of 6 months	Suspected or biopsy proven stage IB-IIIa NSSLc- squamous with coincident bronchial dysplasia or carcinoma in situ in a non- resected region	24	Feasibility
Prostate Phase II NCT01215032, O	1000mg bid for 1 year	Castrate resistant prostate cancer minimum PSA of 2.0 ng/mL	106	PSA response and PSA difference

Organ Site; Phase; ClinicalTrials.gov, EudraCT or ICTRP Number, Status	Study Design	Study Population	Target Accrual	Primary Endpoint
Prostate Phase II NCT01561482, T	500mg bid escalated to 1000mg bid metformin plus 20mg simvastatin qd for 6 months	Biochemical recurrence of prostate cancer with at least 3 PSA rises	37	Improvement in PSA doubling time
Prostate Phase II NCT01243385, O	1000mg bid until disease progression, unacceptable toxicity, or refusal	Biochemical recurrence of locally-advanced or metastatic prostate cancer with at least 3 PSA rises	44	PFS at 12 weeks
Prostate Phase II NCT01620593, R	500mg tid vs. placebo for 1 year	Men with locally-advanced or metastatic prostate cancer planning on castration therapy	94	Metabolic Syndrome PSA response, progression, pathway inhibition in PBMCs
Prostate Phase II NCT02176161, R	750mg bid for 9 months	Prostate cancer patients who have received treatment with radiation or surgery	70	PSA Doubling time
Prostate Phase II NCT01996696, R	500mg tid vs placebo for 3 years	Pathologically confirmed prostate adenocarcinoma with at least 1 high risk feature	104	Mean body weight at 12 months
Prostate Phase II NCT02420652, NO	Metformin bid and aspirin qd vs. placebo for 6 months	Histologically proven prostate cancer treated with local therapy	66	Change in PSA rates
Prostate Pilot NCT02376166, R	850mg bid for 4 weeks	Histologically proven prostate cancer with biochemical progression	15	Completion of telemedicine visits
Solid Tumors Phase II NCT02431676, NO	Self-directed weight loss, coach directed weight loss Vs. up to 2000mg /d metformin	Previous diagnosis of a solid tumor at least 3 months from therapy	120	IGF-1 and IGF-1:IGFBP3 ratio
Thyroid Phase II NCT01341886, C	Levothyroxine with 30% dose reduction with either metformin or placebo for 3 months	Papillary or Follicular thyroid cancer with surgery with levothyroxine treatment	51	TSH

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