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ORIGINAL ARTICLE

A phase I delayed-start, randomized and pharmacodynamic study of metformin and chemotherapy in patients with solid tumors

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Abstract

Purpose Metformin activates AMP-related pathways leading to inactivation of mammalian target of rapamycin (mTOR) and suppression of its downstream effectors, crucial for cancer growth. Epidemiologic studies showed a reduced incidence and improved survival in cancer patients. We conducted a prospective phase I study to assess the safety of metformin in combination with chemotherapy in patients with solid tumors.

Methods We conducted a delayed-start randomized trial of non-diabetic patients in two stages. In Stage 1, we randomized patients to two arms: concurrent arm (metformin with chemo) vs. delayed arm (chemo alone). In Stage 2, patients in delayed arm were crossed over to receive metformin. Patients received metformin 500 mg twice daily with chemotherapy to define dose-limiting toxicities (DLTs) in both stages. Secondary endpoints assessed adverse events (AEs) and response rates. Translational correlates included effects of metformin on expression and phosphorylation of 5′ adenosine monophosphate-activated protein kinase (AMPK) by western blot in PBMCs.

Results A total of 100 patients were enrolled (51 in delayed arm vs. 49 concurrent arm). Rate of DLTs in patients receiving metformin with chemotherapy was 6.1% vs. 7.8% in patients receiving chemotherapy alone. DLTs seen with addition of metformin included those associated with established chemo adverse events. No lactic acidosis or hypoglycemia occurred. Restaging showed stable disease in 46% at cessation of metformin. 28% of patients with measurable tumor markers showed improvement. AMPK phosphorylation showed a four- to sixfold increase in AMPK phosphorylation after metformin.

Conclusions This is the largest phase I study of metformin combined with chemotherapy, which suggests that metformin can be given safely with chemotherapy, and offers a platform for future studies. Post-metformin increase in AMPK phosphorylation may potentially explain lack of disease progression in nearly half of our patients.

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Keywords Metformin · Chemotherapy · mTOR · AMP-activated protein kinase (AMPK) · Biguanide · Anti-diabetic · Diabetes · Cancer

Introduction

Metformin, a biguanide is a relatively safe oral anti-diabetic medication that decreases hepatic glucose production and intestinal glucose absorption, increases insulin sensitivity and sulfonylureas act to stimulate pancreatic islet beta cell insulin release [1]. A study examining all cancer-related mortality among diabetic users of insulin, metformin, and sulfonylureas found that insulin users had a mortality risk almost double that of non-insulin users, and sulfonylurea use was associated with an increased mortality as compared to metformin users [2]. Retrospective case–control and

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cohort studies have also indicated an association between metformin use and decreased nonspecific cancer mortality, higher pathologic complete response rate in breast cancer, and a decreased risk of prostate cancer in diabetic patients [3–5]. In another report, the risk of colon cancer in diabetics was 40–60% higher than that of non-diabetics [6]. While the biological relationship between the two diagnoses is unclear, some studies suggest that altered glucose metabolism, hyperinsulinemia, insulin-like growth factor (IGF-I), and mammalian target of rapamycin (mTOR) may play roles in the pathogenesis of colorectal cancer [7, 8]. These observations triggered the interest among investigators to explore the possible role of metformin in risks of development of malignancy as well as survival.

Preclinical studies have shown antitumor activity of metformin by activating AMP-activated protein kinase, thereby, inhibiting cell growth through cell-autonomous and systemic effects [9, 10]. Metformin inhibits mammalian target of rapamycin complex 1 (mTORC1) and consequently inhibits protein synthesis [11]. Our group at Tufts Cancer Center has also demonstrated that metformin selectively kills cancer stem cells and can function synergistically with chemotherapy, such as doxorubicin [12]. An important link between AMPK/glucose metabolism and colorectal cancer is the observation that Peutz–Jeghers syndrome, a hereditary polyposis syndrome associated with a high risk for gastrointestinal cancers that involve a mutation in the LKB1 tumor suppressor gene, and the finding that LKB1 acts through AMPK for signaling [14]. Metformin is a relatively safe drug for use in diabetic populations over many years and compared to other diabetic drugs, metformin is less likely to causes hypoglycemia by itself [13]. Other known potential adverse events associated with metformin include lactic acidosis [1, 14].

The present study was conducted to evaluate the safety of the addition of metformin to chemotherapy in patients with various malignancies.

Materials and methods

We conducted a delayed-start randomized phase I clinical trial to explore the safety of adding metformin to chemotherapy in non-diabetic patients with different solid cancers [15]. The delayed-start design was attractive since it allows all participants the opportunity to receive the active study drug during the course of trial participation. To determine the safety of adding metformin to chemotherapy, we compared the incidence of dose-limiting toxicities (DLTs) in subjects receiving chemotherapy alone vs. in combination with concurrent metformin. Metformin dose of 500 mg twice daily was chosen based on prior clinical studies involving the use of metformin in non-diabetic patients. The metformin product label indicates a C_{max} of approximately 1.0 µg/ml following a single 500 mg dose in a healthy non-diabetic subject. There is also some accumulation upon repeat dosing. Renal impairment and older age increased metformin exposure, while type-2 diabetics had slightly lower exposure. This study was conducted in compliance with the protocol approved by the Institutional Review Board, and according to Good Clinical Practice standards.

Patient selection

Patients were enrolled if they met the following criteria: histologically or cytologically documented solid malignancy cancer, intended treatment with, or currently being treated by anti-cancer chemotherapy in the adjuvant or advanced setting age 18–79, had Eastern Cooperative Oncology group (ECOG) performance status (PS) ≤ 2, had adequate bone marrow function, adequate renal function (serum creatinine levels < 1.5 mg/dl [males], < 1.4 mg/dl [females]) but if a subject did not meet these criteria, but had an estimated creatinine clearance ≥ 60 ml/min using the Cockroft–Gault calculation, they were allowed, adequate hepatic parameters, including aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels $\leq 2.5 \times$ upper limit of normal (ULN), total bilirubin $\leq 1.5 \times$ ULN, and alkaline phosphatase levels $\leq 2.5 \times$ ULN, anticipated receiving at least three cycles (or treatment periods of at least 3-weeks) of chemotherapy, ability to understand and willingness to sign a written informed consent document. A subject who had previously enrolled on this study was allowed re-enroll if the chemotherapy regimen used was distinct and all other entry criteria were met. Patients with current use of metformin (within 1 week of start of chemotherapy regimen to be assessed), history or states associated with lactic acidosis, alcoholism (acute or chronic), conditions associated with pancreatitis and severe hydration were excluded. However, patients with type 2 diabetes not receiving metformin were allowed; however, they were excluded if there was intent to use metformin for treatment of diabetes during the course of the study.

Study design

As previously described, this was a delayed-start randomized design with a run-in stage to select those patients who were able to undergo chemotherapy alone for one cycle without a DLT [15] (Fig. 1). DLT was defined as any of the following toxicities, related to metformin and/or chemotherapy within the first cycle: Grade 4 absolute neutrophil count (ANC) or Grade 3 thrombocytopenia in the presence of bleeding; Grade 3 or worse non-hematological toxicity of any duration except alopecia (any grade); Grade 3 or 4 nausea/vomiting or diarrhea despite optimal medical management and reversible

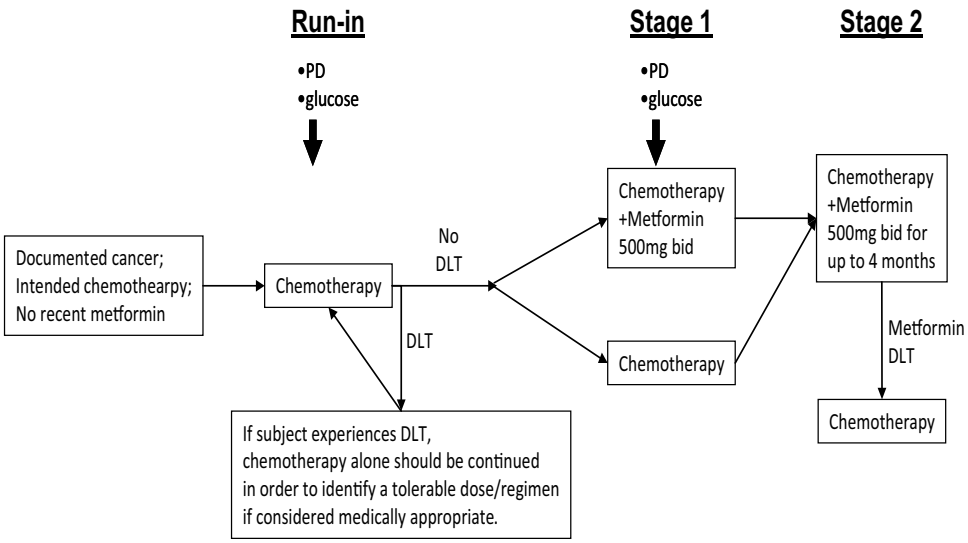

Fig. 1 Delayed start randomization design

laboratory abnormalities with no clinical sequel and/or no clinical significance in the opinion of an Principal Investigator; and any other toxicity that in the view of the Principal Investigator represented a clinically significant hazard to the patient. MTD was defined as the highest dose at which $<33\%$ of six patients experienced a DLT.

For the purpose of this study, a cycle must be at least 3 weeks; if a chemotherapy regimen had cycles that are less than 3 weeks, multiple cycles were consolidated to define 1 cycle (treatment period of at least 3 weeks) for this study. Patients deemed eligible during the run-in stage were randomized with equal allocation to one of the following two treatment arms: (1) metformin arm: patients received the next cycle of chemotherapy with concomitant administration of metformin or (2) delayed metformin arm: patients continued with chemotherapy alone for one cycle and then received metformin on the subsequent cycle.

The duration of Stage 1 in the trial (the single cycle comparing metformin and chemotherapy to chemotherapy alone) was sufficiently long for the effect of metformin and chemotherapy on DLTs to become stably manifest based on documented half-life of metformin (6 h). The half-lives of the common chemotherapy agents used concomitantly were also relatively short given the duration of the first stage (a range of examples include 5 FU, oxaliplatin, and cisplatin half-life ≤ 1 h, doxorubicin, paclitaxel, irinotecan, etoposide half-life $=6$–12 h; sorafenib half-life $=24$ h, and bevacizumab, docetaxel half-life 4–20 days). Given the run-in stage, it was expected that the patient should be at or near "steady state" with respect to any chemotherapy given, so the only change of exposures would be with the administration of metformin, which would be at steady state within 1–2 days. Anecdotally, metformin was given coincidently with chemotherapy very frequently in diabetic patients, without known pharmacokinetic interaction (albeit not rigorously studied).

The investigator caring chose the chemotherapy regimen for each individual subject for the subject based on standard medical practice. Adjustments to chemotherapy dose and schedule were made according to standard practice. Concomitant metformin dose was 500 mg with meals twice daily by mouth, and began on day 1 of the designated chemotherapy cycle. Metformin treatment was discontinued immediately for any subject who experienced a DLT that was considered at least possibly metformin related. Metformin was allowed to resume if preemptive intervention could be implemented that was likely to prevent further DLT. There was no metformin dose modification. Metformin was also discontinued if the chemotherapy regimen was meaningfully changed (other than minor dose modifications or delays) or discontinued. DLT monitoring would extend 4 weeks after the last dose of metformin on study.

Patients were only eligible for randomization in the run-in stage after no DLTs were observed in the prior round with chemotherapy alone. Subjects experiencing DLT with chemotherapy alone continued to be treated with chemotherapy alone at an appropriately adjusted dose/regimen, if medically indicated, until a well-tolerated dose/schedule was identified without DLT. Once a dose/schedule was achieved without the subject experiencing a DLT, then randomization occurred. Randomization was performed using a computer-generated permuted block randomization procedure, with equal allocation to the two study arms. The randomization codes were maintained in an online database and in a separately kept log.

Following the run-in stage, patients were randomly assigned to receive their next cycle of chemotherapy (Stage 1 of the study) with either (1) concomitant metformin or (2) delayed metformin: continuing with chemotherapy alone for one cycle and then receiving metformin on the subsequent cycle. Subjects randomized to the chemotherapy alone arm

who had no further DLT during the first cycle of treatment following randomization were then crossed over to receive metformin concomitantly with chemotherapy for the subsequent cycle (Stage 2 of the study). Subjects randomized to the chemotherapy-alone arm who did not experience a DLT during Stage 1 continued to be treated with chemotherapy alone at an appropriately adjusted dose/regimen, if medically indicated, until a well-tolerated dose/schedule was identified without DLT. Once a dose/schedule was achieved without the subject experiencing a DLT, treatment proceeded to Stage 2 treatment in combination with metformin. In Stage 2, if the subject tolerated treatment well with metformin and their disease had not progressed, then the subsequent cycles of treatment included metformin for up to a total of 4 months. Patients who experienced a metformin-related DLT had their metformin discontinued and might resume planned treatment with chemotherapy if the investigator considered this appropriate and safe. The safety profile among the patients during the first cycle of randomization, with or without metformin, was compared.

An institutional Data and Safety Monitoring Board (DSMB) was utilized, and was responsible for periodic safety review. The committee reviewed safety at least quarterly. Enrollment on the study was to be suspended if three out of the first ten or fewer subjects experience DLT with co-administration of metformin. Additionally, enrollment was to be held if $\geq 30\%$ of more than ten subjects experience a DLT. Upon suspension, the IRB was to be notified and the Safety Committee reviewed safety data. If the safety concern could be attributed to a specific chemotherapeutic agent or regimen, the Safety committee might allow re-opening enrollment either excluding specific subsets of patients or incorporating specific safety measures for the study. These changes were incorporated into a protocol amendment and submitted to, and approved by the IRB prior to re-opening the study to enrollment.

Safety

Adverse events (AEs) were assessed throughout study participation according to standard of care for patients undergoing chemotherapy treatment. An AE is any reaction, side effect or other undesirable event that occurs in conjunction with the use of metformin whether or not the event was considered drug related. All subjects who enrolled on this study were evaluated for AE occurrences. AEs were graded according to the Common Terminology Criteria for Adverse Events (CTCAE) (version 4.0) [16].

Pharmacodynamic studies

Serum glucose was evaluated after at least 1 week of treatment during the initial chemotherapy treatment cycle and after at least 1 week of treatment during the first cycle of randomization. Pharmacodynamic (PD) markers known to be linked to metformin mechanism of action (AMPK signaling pathway) were drawn during a prior chemotherapy-alone cycle, and then during combination therapy with metformin, were evaluated in the Molecular Oncology Research Institute in the laboratory of Dr. Phillip Tsichlis.

Twenty milliliter of blood was collected from patients before and after treatment with metformin in heparinized vacutainer tubes. Peripheral blood mononuclear cells (PBMCs) were isolated by density gradient centrifugation and washed with PBS. Frozen cells were lysed with lysis buffer supplemented with protease and phosphatase inhibitors. Equal amounts of protein were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto polyvinylidene difluoride (PVDF) membranes. The membranes were probed with antibodies specific to phospho-AMPKα (Thr172), total AMPKα, and α-tubulin as a loading control. Quantification of the bands was done using Image J. Intensities of the phospho-AMPKα bands were adjusted to the intensities of the total AMPKα bands, and the intensities of the total AMPKα bands were adjusted to the intensities of the α-tubulin bands to generate adjusted density values. Subsequently, adjusted density values after metformin treatment were divided by the adjusted density values before the metformin treatment to generate the ratio of phospho-AMPKα or total AMPKα after metformin to before metformin. Ratio greater than 1 will indicate an increase in the phosphorylation of AMPKα or total level of AMPKα after metformin treatment, and ratio lesser than 1 will indicate a decrease in the AMPKα phosphorylation or AMPKα level after treatment with metformin [17–19].

Efficacy

Tumor measurements were performed at baseline and in 8-week intervals. Tumor responses were evaluated based on RECIST 1.1 by the investigators [20].

Statistical analysis

Given patient accrual rates at our center and collaborating centers, we expected to be able to evaluate 100 patients in total (50 per group). Given an expectation that 15% of subjects might be lost-to-follow up or withdraw from the study prior to completion of Stage 1, we aimed to recruit a total sample size of 118 (59 per study arm) to safeguard against losses of subjects. For the primary outcome of incidence of DLT during the first cycle following establishment of chemotherapy tolerance, we set a non-inferiority margin of 20% based on the typical threshold for defining the MTD (30% of patients have DLT). The expected rate of DLT in

the two groups in subsequent cycles of treatment was difficult to estimate given the lack of published estimates in the literature for this specific endpoint, and given the heterogeneity of regimens and diagnoses for enrolled patients. A generally accepted upper boundary for DLT rate during the first cycle of chemotherapy is 20–33%, as this is often used to define the MTD in phase 1 studies across a wide range of malignancies and chemotherapy regimens. In this study, the rate would be considerably lower than that due to the requirement that the preceding cycle is DLT-free. Yet the rate would not be vanishingly small, since the significant majority of patients eventually do develop a DLT on therapy. Therefore, a rate of 10% was chosen as the estimate around which to size the study. With 50 patients per group (total sample size of 100), and assuming 10% incidence of DLT in the metformin group and also in the delayed metformin group, we had 94% power to claim non-inferiority of metformin as compared to the chemotherapy-alone delayed metformin group at the 0.05 level. If however, the DLT rate was actually 15% in each arm, we would have reduced power for this analysis (69%). Continuous measurements were summarized by mean (± SD) or median. Categorical data were summarized by frequency and percentages. Progression-free survival (PFS) analysis used the Kaplan–Meier method. PFS was calculated from the first dose until PD according to RECIST 1.1 or death. A descriptive listing of all measured PD parameters, including serum glucose, AMPK, was prepared.

Results

Patient population

Adult patients aged 18–79 years with histologically confirmed solid cancer who were undergoing treatment with chemotherapy for their cancer either adjuvant or systemic therapy for advanced disease were evaluated. Among the 100 subjects, there were 16 different malignancies. Breast, pancreatic and colorectal cancers were the most common. Metformin was evaluated in addition to either monotherapy or combination chemotherapy regimens (Table 1). For individual subjects, participation in the trial lasted until metformin is discontinued. A maximum of 4 months from the initiation of metformin treatment was allowed.

Table 1 Patient demographics

	Concurrent ($n=48$)	Delayed ($N=52$)
Sex = no (%)		
Male	25 (52%)	29 (56%)
Female	23 (48%)	23 (44%)
Primary cancer		
Breast	9	12
Prostate	6	2
Colorectal	14	5
Lung	2	3
Lymphoma	3	0
Gastroesopageal	3	5
Nasopharyngeal	2	2
Brain	4	4
Pancreas	3	8
Unknown primary	1	1
Appendiceal	0	1
Cholangiocarcinoma	0	2
Myeloma	0	2
Liver	0	1
Ovarian	1	1
Renal	0	3
Chemo agents		
Oxaliplatin	4	2
Capecitabine	9	11
5-FU	5	4
Temozolomide	4	2
Epirubicin	2	1
Pemetrexed	0	1
Bevacizumab	5	6
Carboplatin	0	3
Gemcitabine	3	7
Erlotinib	0	1
Navelbine	0	2
Docetaxel	2	0
Doxorubicin	3	1
Abiraterone	3	1
Irinotecan	5	4
Cetuximab	2	0
Bortezomib	0	2
Paclitaxel	3	2
Fulvestrant	1	1

Safety and tolerability

The rate of DLTs in patients receiving metformin in addition to chemotherapy was not higher than the DLTs in patients receiving chemotherapy alone (8.3% vs. 11.1% in Stage 1) (Table 2). DLTs associated with established chemotherapy AEs include vomiting and dehydration with metformin plus irinotecan/5-FU, and proteinuria with metformin plus bevacizumab. No *lactic acidosis*, a known AE associated with metformin, occurred in any patient. Adverse events ≥ Grade 3 that were not related to metformin were varied, with a higher incidence in the concurrent arm (8.3 vs. 5.5%) in Stage 1 but a lower incidence in Stage 2 (11.1% vs. 16.7%) (Table 3). One

patient on metformin with gemcitabine suffered an unrelated fatality, an intracranial hemorrhage from a cerebral metastasis.

Efficacy

Restaging showed stable disease in 46% at cessation of metformin. 28% of patients with measurable tumor markers showed improvement.

Pharmacodynamic analyses

The effect on glucose levels varied. Although lower mean glucose levels were documented in Stage 2, the addition of metformin to chemotherapy was not associated with hypoglycemia in these non-diabetic cancer patients (Table 4).

To monitor whether the amount of metformin used in the study was pharmacologically effective, PBMCs isolated from 42 patients before and after treatment with metformin were subjected to Western blotting (Fig. 2). Phosphorylation of AMPKα was analyzed relative to the levels of total AMPKα and levels of AMPKα were analyzed relative to the

Table 2 Dose-limiting toxicities

	Run-in stage Chemo (C) w/out DLT	Stage 1 Chemo + metformin (C + M) vs. Chemo (C)	Stage 2 Chemo + metformin (C + M)
Concurrent	*N* = 43 1 = G3 fatigue (2.3%)	*N* = 36 3 = G3 anemia, ↓albumin, ↑ALT (8.3%)	*N* = 27 N/A
Delayed	*N* = 44 2 = G3 thrombocytopenia, HFS (4.5%)	*N* = 36 4 = G3 syncope, dehydration, ↑bilirubin (11.1%)	*N* = 24 2 = G3 dehydration, vomiting, proteinuria (8.3%)

HFS Hand–foot syndrome, *G* grade, ↓ decrease, ↑ increase, *ALT* alanine aminotransferase

Table 3 Adverse events ≥ 3

	Run-in stage Chemo (C) w/out DLT	Stage 1 Chemo + metformin (C + M) vs. Chemo (C)	Stage 2 Chemo + metformin (C + M)
Concurrent	*N* = 43 1 = G3 HFS (2.3%)	*N* = 36 1 = G3 PTE, DVT 1 = G3 neuropathy 1 = G3 anemia, hypoalbuminemia (8.3%)	*N* = 27 1 = G3 hypokalemia 1 = G2 infusion reaction 1 = G3 thrombocytopenia (11.1%)
Delayed	*N* = 44 1 = G3 thrombocytopenia (2.2%)	*N* = 36 1 = G3 hemoptysis 1 = syncope (5.5%)	*N* = 24 1 = G3 proteinuria 1 = G3 mucositis 1 = G3 dehydration 1 = G4 neutropenia (16.7%)

G grade, *PTE* pulmonary thromboembolism, *DVT* deep venous thrombosis

Table 4 Glucose levels

	Run-in stage Chemo (C) w/out DLT	Stage 1 Chemo + metformin (C + M) vs. Chemo (C)	Stage 2 Chemo + metformin (C + M)
Concurrent	*N* = 43 Mean: 109 Range: 67–180	*N* = 36 Mean: 104 Range: 65–168	*N* = 27 Mean: 102 Range: 70–178
Delayed	*N* = 44 Mean: 111 Range: 64–235	*N* = 36 Mean = 111 Range: 47–308	*N* = 24 Mean: 99 Range: 67–131

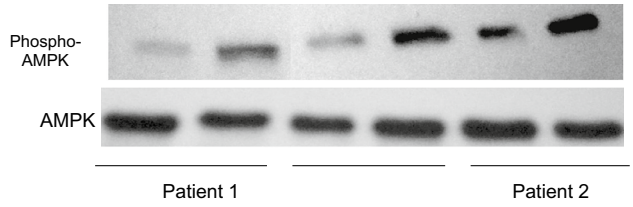

Fig. 2 AMPK phosphorylation in PBMCs before and after metformin

levels of α-tubulin. Gel band density analysis of phospho-AMPKα showed that in 50% cases (21 out of 42 patients subjected to the analysis) phosphorylation of AMPKα was increased after metformin (the ratio of phospho-AMPKα after metformin to before metformin is greater than 1) with the mean = 1.114 (± 0.512) (Fig. 3). Analysis of total levels of AMPKα showed similar results. In 50% cases (21 out of 42 patients) levels of AMPKα protein were increased after metformin (the ratio of total AMPKα after metformin to before metformin treatment is greater than 1) with the mean = 1.04 (± 0.28) (Fig. 4).

Discussion

This is the largest prospective phase I study that enrolled 100 non-diabetic patients with different cancers and showed that metformin can be given safely with chemotherapy. Preclinical evidence support enhanced elimination of tumors when chemotherapy is combined with metformin. Many diabetic patients have received metformin during administration of chemotherapy, with no specific safety contraindication for concomitant administration [21, 22]. Additionally, there is safety experience with administering metformin to non-diabetic patients [1, 23]. However, clinical safety of combining

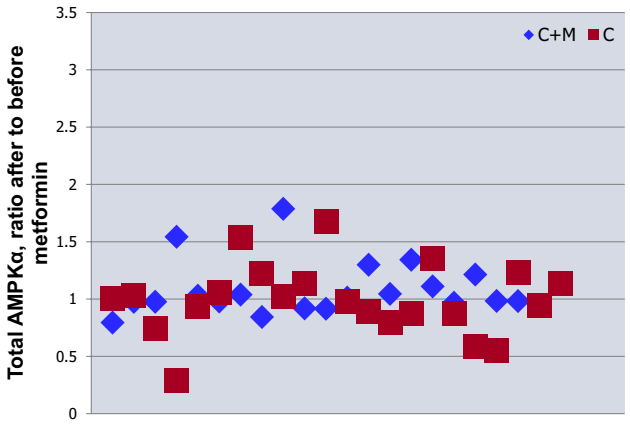

Fig. 3 Analysis of total levels of AMPKα. Analysis of total levels of AMPKα showed that levels of AMPKα protein were increased in 50% cases (21 out of 42 patients) after metformin treatment

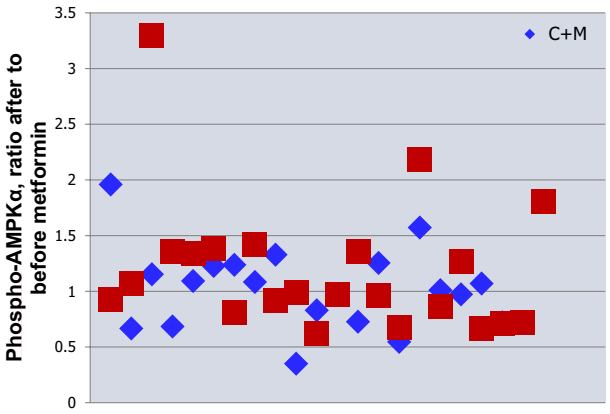

Fig. 4 Effect of metformin on phosphorylation of AMPKα. The levels of AMPKα were also decreased in 50% cases (21 out of 42 patients) after metformin treatment

metformin with chemotherapy was not previously tested in a prospective study in either diabetic or non-diabetic patients. This study provides an initial evaluation of the feasibility and safety of combining metformin with chemotherapy. Moreover, our study design also had the advantage of establishing tolerance of the chemotherapy regimen prior to administering concomitant metformin [15].

There is a wide range of chemotherapy regimens that can potentially be combined with metformin. This protocol was designed to gain broad experience across multiple malignancies and chemotherapy regimens. The influence of the heterogeneity of agents and regimens was partially controlled for by prospectively establishing that a regimen was well tolerated in each subject before adding metformin to the combination. Administering the chemotherapy regimen to a subject for at least one cycle in the absence of DLT before concomitant metformin was mandated. If a DLT occurred, the regimen was modified or supportive care implemented. The modified regimen was then re-administered to the subject to confirm that the adjusted regimen was indeed well tolerated in that subject prior to initiating concomitant metformin treatment.

In accordance with the delayed-start design, we conducted three analyses. These analyses were divided into a primary analysis and secondary analyses because there were a number of competing causes of adverse events over time, including cumulative toxicity from chemotherapy and mounting risk of disease progression despite chemotherapy.

As a primary analysis, we tested for the non-inferiority of concomitant administration of metformin with chemotherapy to chemotherapy alone during Stage 1, as previously described in the statistical analysis section. A secondary analysis was conducted using the non-inferiority methods described above that compares the incidence of DLT among patients in Stage 2 of the delayed metformin arm with the

frequency of DLTs experienced by patients in the delayed metformin arm during Stage 1. A third analysis was conducted to compare the incidence of DLTs among all patients receiving metformin (in Stage 1 and delayed in Stage 2) with the incidence of DLTs among patients not receiving metformin in Stage 1. The results revealed that the rate of DLTs in patients receiving metformin in addition to chemotherapy was not higher than the DLTs in patients receiving chemotherapy alone (8.3% vs. 11.1% in Stage 1). DLTs associated with established chemotherapy AEs include vomiting and dehydration with metformin plus irinotecan/5-FU, and proteinuria with metformin plus bevacizumab. No lactic acidosis occurred in any of the 100 patients enrolled in the study. Adverse events ≥ Grade 3 that were not related to metformin were varied, with a higher incidence in the concurrent arm (8.3 vs. 5.5%) in Stage 1 but a lower incidence in Stage 2 (11.1% vs. 16.7%).

An extra advantage of metformin noticed in this study was the absence of any symptomatic hypoglycemia [21]. Though, the effect on glucose levels varied. But the lower mean glucose levels documented in Stage 2 upon the addition of metformin to chemotherapy were not associated with hypoglycemia in these non-diabetic cancer patients. Translational correlates included effects of metformin on expression and phosphorylation of AMPK by western blot in PBMCs and showed Gel band density analysis showed an increased phosphorylation of AMPKα was following after metformin treatment and total levels of AMPKα showed similar results. These results suggest post-metformin increase in AMPK phosphorylation may potentially explain lack of disease progression in nearly half of our patients.

MPK is a cellular energy sensor that is conserved in eukaryotes. Elevated AMP/ATP ratio activates AMPK, which inhibits energy-consuming processes and activates energy-producing processes to restore the energy homeostasis inside the cell [24]. Metformin, commonly used for the treatment of type II diabetes is also an AMPK activator. AMPK may act to inhibit tumorigenesis through regulation of cell growth, cell proliferation, autophagy, stress responses and cell polarity [25]. Metformin induces LKB1 tumor suppressor phosphorylates and activates AMPK when cellular energy levels are low, thereby suppressing growth through multiple pathways, including inhibiting the mTORC1 kinase that is activated in the majority of human cancers, indicating that these compounds could be used to suppress growth of tumor cells [26, 27].

Although chemotherapeutic regimens often retard tumor growth, there is a high variability in the responses to these agents in cancer patients as well as resistance to these drugs is very common resulting in tumor recurrence or progression. Once explanation to this phenomenon is related to the CSC hypothesis suggesting that tumors contain a small number of tumor-forming, self-renewing CSCs within a population of non-tumor-forming cancer cells [28, 29]. Contrary to most cells within the tumor, these CSCs are resistant to chemotherapy, and being pluripotent, can regenerate all the cell types in the tumor through their stem cell-like behavior following treatment. As previously described by our group that metformin also selectively kills CSCs and acts synergistically with chemotherapeutic agents, such as doxorubicin to reduce tumor growth and prolong remission [12].

Another scintillating challenge associated with the chemotherapeutic drugs is the toxicities, such as neutropenia, anemia, fatigue, alopecia, nausea, vomiting and anorexia. This factor is further complexed by the fact that reduced l doses of these chemotherapeutic agents are usually not effective in suppressing tumor growth. This underlines that need for exploring agents that can be combined with lower doses of the existing chemotherapeutic drugs without the cost of losing efficacy. Hanna et al. showed that the addition of metformin to paclitaxel resulted in an increase in the number of cells arrested in the G(2)-M phase of the cell cycle, and decreased the tumor growth and increased apoptosis in tumor-bearing mice, when compared with individual drug treatments [30].

In brief, metformin is a long-approved drug with excellent safety record, and our study suggests the feasibility of combining with both cytotoxic and targeted agents in patients with solid tumors. Our data in addition to both epidemiological and preclinical data warrant further investigation in future studies and its relation to metabolomics.

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Compliance with ethical standards

Conflict of interest The author(s) declare that they have no competing interests.

Ethical approval All the procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee at Tufts Medical Center, Boston, MA and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

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