DR. SHUCHI GULATI (Orcid ID : 0000-0001-6793-9694)

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TITLE PAGE

Title: Phase-I dose-finding study of metformin in combination with concurrent cisplatin and radiation in patients with locally advanced head and neck squamous cell cancer

Authors: Shuchi Gulati[a], MD, Janki Desai[b], M.Pharm, Sarah Palackdharry[a], MS, John C. Morris[a], MD, Zheng Zhu[c], PhD, Roman Jandarov[c], PhD, Muhammad K. Riaz[a], MD, Vinita Takiar[d], MD, PhD, Michelle Mierzwa[e], MD, J. Silvio Gutkind[f], PhD, Alfredo Molinolo[g], MD, PhD, Pankaj B. Desai[b], PhD, Nooshin Hashemi Sadraei[a], MD, and Trisha M. Wise-Draper[a*], MD, PhD

Affiliations:

[a] Department of Internal Medicine, Division of Hematology/Oncology, University of Cincinnati College of Medicine, Cincinnati, OH

[b] Division of Pharmaceutical Sciences, College of Pharmacy, James L. Winkle College of Pharmacy, University of Cincinnati, Cincinnati, OH.

[c] Department of Environmental Health, University of Cincinnati College of Medicine, Cincinnati, OH

[d] Department of Radiation Oncology, University of Cincinnati College of Medicine,

Cincinnati, OH

[e] Department of Radiation Oncology, University of Michigan, Ann Arbor, MI

[f] Department of Pharmacology, UC San Diego Moores Cancer Center, La Jolla, CA

[g] Department of Pathology, UC San Diego Moores Cancer Center, La Jolla, CA

* Correspondence should be addressed to:

Trisha Wise-Draper M.D., PhD

3125 Eden Ave ML 0562

Cincinnati, OH 45267 USA

Tel: 513-558-2826/Fax: 513-558-6703

E-mail address: wiseth@ucmail.uc.edu

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Phase I metformin and CRT in head and neck cancer

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Author Contributions

Trisha M. Wise-Draper: Conceptualization, methodology, supervision, visualization, validation, writing (review and editing), funding acquisition. **Shuchi Gulati:** Conceptualization, methodology, visualization, validation, writing (original draft), writing (review and editing), funding acquisition. **Vinita Takiar, J. Silvio Gutkind, John C. Morris, Janki Desai, Pankaj M. Desai**: **Muhammad K. Riaz, John C. Morris, Michelle Mierzwa, Nooshin Hashemi Sadraei, Sarah Palackdharry, Alfredo Molinolo:** Formal analysis, project management, supervision, validation, writing (review and editing). **Zheng Zhu, Roman Jandarov:** Formal statistical design and analysis, writing (review and editing)

Precis: Metformin is an oral drug, commonly used to treat patients with type-2 diabetes mellitus. In this phase I trial dose escalation clinical trial, we tested the safety of combining metformin with chemo-radiation therapy in locally advanced head and neck cancer.

ABSTRACT

Purpose: Five-year overall survival (OS) remains 50% for locally advanced head and neck squamous cell carcinoma (LAHNSCC) underscoring the need for improved treatments. Anti-diabetic agent, metformin, improved survival in HNSCC patients in retrospective studies. Therefore, we conducted a phase I dose escalation study combining metformin with chemoradiation (CRT) in LAHNSCC.

Methods: Non-diabetic LAHNSCC patients were enrolled to receive escalating doses of metformin and CRT based on the modified toxicity probability interval (mTPI) design. Metformin cohort doses included 2000, 2550, 3000mg daily in divided doses in addition to cisplatin (100mg/m2 days 1, 22, 43) and standard radiation (70Gy). Adverse events (AEs) were categorized per CTCAE v4.03.

Results: Twenty patients were enrolled (two withdrew consent). Median age 56 years; majority were male (83%), Caucasian (88%), p16 positive (72%) and tobacco users (61%). Median length of metformin exposure was 28.5 days. Most common grade ≥ 3 toxicities were nausea (11%), vomiting (11%), mucositis (6%), acute kidney injury (AKI) (17%), anemia (6%), and leukopenia (11%). Dose limiting toxicities included diarrhea and AKI. After median follow-up of 19 months, 2-year OS, and progression free survival (PFS) were 90% and 84% respectively. No hypoglycemia events or lactic acidosis were observed. Cisplatin administration did not affect metformin pharmacokinetics. The maximum tolerated dose (MTD) for metformin could not be determined given limited number of patients who tolerated metformin during CRT.

Conclusion: This is the first phase I trial combining metformin with CRT. OS/PFS were

encouraging in this limited patient population warranting further investigation in a phase II trial.

Keywords: metformin, phase I, clinical trial, head and neck cancer

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INTRODUCTION

According to the Surveillance, Epidemiology, and End Results (SEER) database, approximately. 65,000 new cases of head and neck cancer (HNC) are estimated to be diagnosed in the United States in 2019 with nearly 16,500 patients deaths this year.[1] Early stage HNSCC tumors are often curable with single modality treatment. However, approximately 60% of newly diagnosed patients present as LAHNSCC (AJCC8 stage III or IV).[2] For patients that are unable to undergo surgery or prefer organ preservation,

available treatment options include concurrent chemotherapy and radiation (CRT) or sequential induction chemotherapy followed by radiation.[3] In a randomized phase III trial (RTOG 0522) comparing radiation therapy plus cisplatin with or without cetuximab for stage III to IV HNSCC patients, the 2-year PFS was ~65% and the 2-year OS was ~80%.[4] Moreover, a previous meta-analysis of randomized controlled trials has demonstrated that adding chemotherapy to radiation therapy does improve PFS and OS, but with only 50% 5 year survival,[4,5] underscoring the need for improved regimens.

Metformin (1,1-dimethylbiguanide hydrochloride) is an oral drug commonly used to treat patients with type-2 diabetes mellitus, polycystic ovary syndrome and metabolic syndrome.[6] Animal models have proven metformin to work by inhibiting the mTOR pathway along with an increase in the phosphorylation of 5' AMP-activated protein kinase (AMPK), leading to an anti-tumor effect.[7] Epidemiological studies, which analyzed either all HNC patients or those with laryngeal cancer, have demonstrated that diabetic patients treated with metformin not only presented with earlier stage disease, but had better outcomes when compared to diabetic patients not administered metformin.[8,9] Rego et al. conducted a systematic review showing that metformin causes cell cycle arrest in G0/G1 phase, and apoptosis of cancer cells giving insight into possible mechanisms of metformin mediated anti-cancer effects.[6] In addition, a “window of opportunity trial” where metformin was administered to patients between biopsy and definitive surgical resection at 1,000 mg twice daily, showed metformin promoted apoptosis as well as increased stromal markers of metabolism (eg. CAV1 and GALBG).[10] Metformin’s impact on the metabolic milieu of the tumor microenvironment; especially its ability to reduce the level of hypoxia is of special interest and has been explored in preclinical studies.[11, 12] This

metabolic effect of metformin is also thought to be the mechanism by which sensitivity of tumor tissue to PD-1 blocking drugs increases, laying the foundation for clinical trials combining metformin with immunotherapy drugs such as pembrolizumab.[13] In lung cancer cell lines metformin has also been shown to be an effective radiosensitizer.[14] Given the anti-cancer properties of metformin due to its metabolic effects as well as its radiosensitization effects, we conducted a phase I open-label single-site dose escalation study combining metformin with CRT in LAHNSCC.

METHODS

Patients

Eligible patients were ≥ 18 years old with histologically or cytologically confirmed newly diagnosed locally advanced stage III/ IV HNSCC (T1-2, N2a-3 or T3-4 AJCC 7th edition), Eastern Cooperative Oncology Group (ECOG) performance status ≤ 1, and had adequate organ function. Exclusion criteria included patients with nasopharyngeal cancer, metastatic disease, diabetes requiring insulin or on metformin within the last 4 weeks, history of other active intercurrent illnesses (eg. significant cardiovascular disease, viral infections or major psychiatric illness), and patients receiving medications with potential to induce lactic acidosis.

Study Design and Treatment

This was a single-center, open-label, non-randomized phase-1 dose-escalation trial of metformin in combination with CRT in previously untreated LAHNSCC. The clinical trial was registered on ClinicalTrials.gov (NCT02325401). The study was approved by the institutional review board at the University of Cincinnati and was conducted in accordance

with Good Clinical Practice guidelines and the Declaration of Helsinki. Written informed consent was received from all participating patients prior to enrollment.

Radiation:

All patients were treated with standard of care radiation, which entailed 70 Gy to gross disease with margin and a simultaneous integrated boost of 56 Gy to areas at risk for microscopic disease, all in 35 fractions. An optional, intermediate dose volume of 63 Gy was allowed based on physician discretion to entail areas felt to be at higher risk for recurrence. As all patients were treated with radiation in the same fashion, the radiation is believed to have had no bearing on outcomes or metformin-related toxicity.

Chemotherapy:

Patients were treated with institutional SOC bolus cisplatin (100mg/m2 days 1, 22 and 43) with intravenous hydration on day of treatment and 2 days subsequently. Mannitol was added for those with central line access. Antiemetic pre-medications were used including a 5-HT3 antagonist, corticosteroids and fosaprepitant. For acute nausea and vomiting, supportive care was at the discretion of the treating investigator.

Metformin Dose Escalation Phase:

Cohorts of patients received escalating doses (2000, 2550 or 3000mg divided in daily doses) of metformin with a 7-14 day lead-in prior to CRT based on mTPI design[15] (**Fig. 1**) to allow for possible re-escalation after previous de-escalation and maximizing the ability to identify the MTD. Patients remained on metformin for the duration of CRT as tolerated. The study was initially designed to allow for a 7-day lead-in of metformin where metformin was increased after 3 days to full cohort dose, but due to nausea with accelerated escalation to full cohort dose, the study was amended (patient 8 onward) to

allow for a lead-in of 14 days and slower escalation to cohort dose. During the lead-in period, patients were started on oral metformin 500mg twice a day (BID) on day -14 (day -7 for initial amendment) (**Fig. 1**). They were then continued on this dose or on day -3 escalated to their final cohort dose three times a day (TID). Starting dose of metformin was 2000mg daily in divided doses and the highest dose was 3000mg daily. Metformin was taken with meals and held for any contrast scans for 48 hours. Patients were instructed to check blood glucose on a daily basis. Metformin was also held for creatinine above 1.5 mg/dL or glomerular filtration rate (GFR) of <30 mL/min.

Dose-limiting toxicity (DLT)

DLT was defined as the appearance of side effects during treatment that were severe enough to prevent further increase in dosage of metformin, or to prevent continuation of SOC treatment at any dosage level. DLTs were defined as metformin-related grade 3 or 4 non-hematologic toxicities other than alopecia, nausea or vomiting, or those that the investigator determined were possibly, probably, or definitely related to metformin rather than SOC. Patients were considered evaluable for toxicity if they completed 3 or more days of metformin. DLTs were assessed until 2 weeks after completion of radiation.

Three patients were enrolled into a cohort. The frequency of DLTs per cohort determined which cohort the following patients would enroll (**Fig. 1**). If no DLTs were identified, then up to three patients were enrolled at the next cohort level. However, if two total DLTs were experienced in any cohort level at any time during the process, the following patients were to be enrolled into cohorts of one patient each until DLTs were assessed for all patients. The phase-I portion was not considered complete until all 18 evaluable patients

had been enrolled or the number of observed DLTs had resulted in an unacceptable dose at the lowest dose level.

Assessments

The primary end point of this study was to determine the MTD of metformin in combination with CRT for LAHNSCC. Key secondary end points included evaluation of AEs (graded using CTCAE v4.03 criteria); evaluation of PFS and OS at 2 years, and the effect of cisplatin pharmacokinetics on the tolerability of metformin. Safety and tolerability assessments were conducted at study visits, at end of treatment, and during follow-up. After completion of treatment, patients were followed monthly for the first 3 months; every 3 months for the first year followed by every 6 months for 2 years for survival until death or withdrawal of consent. Patients were required to undergo a PET/CT at 12 weeks to document treatment response.

Pharmacokinetics (PKs)

For PK analysis, serial blood samples were collected at 0, 0.5, 1, 2, 4 and 24 hours after metformin administration on days -7 or -14 and 1. Metformin plasma levels were quantitated using a LC/MS method. ThermoScientific LTQ-FT, a hybrid mass spectrometer consisting of a linear ion trap and a Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer were used for the detection of metformin. Metformin was separated from other analytes by reverse phase chromatography using a Waters XBridge Amide column (2.1 x 100 mm, 3.5 µm). The composition of mobile phase was 25:75 [95:5 water: acetonitrile 0.1% HCOOH]: [95:5 acetonitrile: water 0.1% HCOOH]. Metformin and the deuterated internal standard Metformin-d6 Hydrochloride (Toronto Research Chemicals, Canada) were detected by multiple reaction monitoring of

the 130.11 m/z → 60 m/z and 136.15 m/z →60 m/z quantifying transitions for metformin and Metformin-d6 Hydrochloride, respectively. PK analysis was done using Non–Compartmental Analysis (Phoenix® 64 WinNonlin®, Certara USA, Inc.).

Correlative Pharmacodynamics

Blood samples were obtained for pharmacodynamics (PD) as shown in **Fig. 1**. Samples were processed by a CLIA certified clinical laboratory for determination of glucose, lactate levels (to document any signs of developing lactic acidosis), vitamin B12 (given that long term use of metformin has been associated with low vitamin B-12 levels),[16] and C-peptide levels (to study impact on pancreatic beta cells).[17]

Statistical Analyses

The dose finding part of the study was to enroll a maximum of 18 evaluable patients. The sample size was chosen based on the maximum number of patients that could have been enrolled on a 3+3 design studying three doses. Secondary end points of safety assessed by clinical review of AEs and laboratory tests were analysed with appropriate summary statistics. Any patient who had received ≥3 days of metformin was considered evaluable for toxicity. Secondary efficacy end points (OS and PFS) were analysed via Kaplan-Meier summary statistics. PFS was defined as the duration of time from start of treatment to time of progression and OS was the duration of time a patient was alive from start of treatment until time of death. Comparisons of Kaplan-Meier survival data between different subgroups were conducted by log-rank test. A p-value less than 0.05 indicates significant difference between the subgroups. For correlative pharmacodynamics analysis, patients' glucose, vitamin B12, and c-peptide were measured before metformin, pre-chemo, and off metformin. Measurements (pre-chemo,

and off metformin) were compared with before metformin using paired two sample t-tests. The data cut-off for these analyses was October 22, 2018. All statistical analyses were performed using R software (The R project for statistical computing), version 3.3.3.

RESULTS

Patient Characteristics and Disposition

Between May 11, 2015 and December 26, 2017, 21 patients were consented with 20 patients enrolled. Two patients withdrew consent during metformin lead-in period due to non-DLT related reasons. The remaining 18 patients were included for analysis. Median age of the cohort was 56 years (range 46-65 years); majority of the enrolled patients were male (83%), and Caucasian (88%). 61% of the patients were tobacco users (> 10 pack years). Thirteen patients tested positive for p16 expression (remaining 5 were either negative or did not have p16 completed) **(Table 1).** In 50% of patients, metformin was discontinued or interrupted due to an adverse event; a total of 10 patients were able to stay on metformin during CRT for a sufficient period of time (>half the duration of CRT).

Dose escalation, Dose Limiting Toxicities, and Maximum Tolerated Dose

Because of early nausea in the lead-in period, the protocol was amended after patient 7 to allow slower escalation of metformin to cohort dose. Of the evaluable patients, 85% experienced one or more AEs **(Table 2).** Most common AEs were related to the gastrointestinal tract (GIT) with nausea seen in 77% of the patients followed by vomiting and diarrhea. DLTs included grade 3 diarrhea (cohort 3) and AKI (cohort 2), the latter of which occurred prior to initiation of CRT. Most common grade ≥ 3 toxicities were diarrhea (6%), nausea (11%), vomiting (11%), mucositis (6%), acute kidney injury (AKI) (17%),

anemia (6%) and leucopenia (11%); most of which were related to SOC treatment rather than metformin. Lactic acidosis was not observed. Based on the mTPI design, MTD for metformin could not be reliably determined given the limited number of patients who tolerated the drug during CRT. However, 2550 mg daily in combination with CRT, was the highest dose tolerated at the highest frequency suggesting this dose may be acceptable as a recommended phase 2 dose (RP2D).

Survival Outcomes

After a median follow up of 19 months; 1-year OS and PFS were 90%. 2-year OS and PFS were 90% and 84% respectively (**Figure 2**). Two deaths were reported during the course of follow-up. One patient had a sudden unexplained death within 3-4 days of completion of CRT. The patient had received only 5 days metformin lead-in, while the other died 29 months after completion of treatment. Both deaths were deemed unrelated to metformin and unlikely related to disease. One patient experienced disease recurrence 17 months after completion of CRT and underwent salvage resection and is subsequently free of disease.

Pharmacodynamics

Blood samples were collected prior to receiving metformin (Day -7 or -14), after metformin lead-in but prior to CRT (Day 1), and after completion of therapy. Vitamin B12, glucose, C-peptide and lactate levels were measured. No elevations in lactate concerning for lactic acidosis were observed. Additionally, glucose levels did not decrease significantly during treatment with metformin. An increase in C-peptide was observed after metformin lead-in in most patients that returned to baseline after treatment, but changes were not statistically significant (**Figure 3**). Importantly, vitamin B-12 levels were

decreased by metformin administration in most patients requiring supplementation in one patient although not statistically significant (**Figure 3**). Interestingly, the vitamin B-12 levels returned to above baseline after metformin treatment was completed (p value < 0.001).

Archival tissue immunohistochemistry analysis revealed activation of the mTOR pathway suggesting that these patients would benefit from mTOR pathway inhibitors like metformin (**Supplemental Fig.1 and Table 1**).

Pharmacokinetics

Metformin is primarily excreted unchanged by the kidney with a mean renal clearance (CL_R) of 500mL/ min. This indicates that CL_R of metformin is higher than GFR, and active tubular secretion is the principal mechanism of metformin elimination.[18] Cisplatin is well known to cause nephrotoxicity.[19] Approximately, 50 percent of the cisplatin administered is excreted renally in the first 24 hours. As such, the concentration of platinum achieved in the renal cortex is several-fold greater than that in plasma and other organs.[20] Cisplatin primarily injures the S3 segment of the proximal tubule, causing a decrease in the GFR.[21] In fact, its adverse effect on kidney function is one of its primary dose-limiting toxicities. Thus, in this combination study the systemic exposure (plasma levels and the overall Area Under the Curve or AUC) of metformin may have been considerably impacted by the co-administration of cisplatin. The nephrotoxic effects of cisplatin could potentially reduce metformin CL_R resulting in an increase in the systemic exposure. Therefore, for those patients for which sufficient blood samples were collected, we performed pharmacokinetics at multiple time-points. It was observed that the AUC on day 1 did increase with escalation of metformin dose as would be expected

(6181 +/- 4340 ng/ml*hr in cohort 1 compared to 8499 +/- 3032 ng/ml*hr in cohort 3) **(Table 3).** Although, there was substantial patient inter-subject variability, importantly, the creatinine clearance did not seem to affect acute concentrations of metformin in these patients. Longer term effects were unable to be analyzed in this study.

DISCUSSION

We present the results of a phase-1 dose-finding clinical trial of metformin added to CRT in LAHNSCC patients. Metformin has been suggested to decrease not only the rates of loco-regional recurrences but also overall survival of HNSCC patients in previous retrospective studies and systematic reviews.[24] Several studies have demonstrated potential safety and efficacy of metformin in cancers other than HNSCC including rectal cancer, acute lymphoblastic leukemia, and lung cancer[25262728]. We conducted the first phase-1 clinical trial to evaluate metformin combined with CRT in LAHNSCC patients.

MTD for metformin cannot be reliably established from this study given that only 10 of the evaluable 18 patients were able to tolerate metformin through a significant portion of CRT. However, 2550mg daily was the highest dose that appeared to have the most tolerable profile in this study suggesting it may be the most reliable dose for subsequent phase II studies. Given that DLTs were to be collected until 2 weeks after completion of radiation, this does limit the reliability of our full assessment of DLTs with the combination. Patients experienced DLTs at dose levels 2 and 3. Side effects were predominantly involving the GIT and kidneys and were typically dose related. No deaths related to metformin were reported in the trial. Overall the combination was safe but with limited tolerability with CRT due to GI toxicity.

The small number of patients included in this trial and the design of the trial limits drawing conclusions about the efficacy of metformin. Nevertheless, the 2-year OS and PFS of 90% and 85% respectively, are improved when compared to historical controls (of 80% and 65% respectively). This could however be influenced by the high p16 positive prevalence in our patient population, which may have confounded the results. Metformin warrants further investigation in a phase II trial, but would be better tolerated in combination with less emetogenic systemic therapies.

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FIGURE LEGENDS

Figure 1. A. Clinical Trial Schema. Eligible patients with locally advanced (AJCC7 Stage III/IV) HNSCC were consented to receive metformin during a lead in period of 7 (patients numbered 1 to 7) or 14 (patients 8-onward) days. The dose was escalated slowly to allotted cohort dose (**B)** over the lead-in period and then continued at cohort dose during CRT. Starting dose of metformin was 2000mg daily in divided doses in addition to cisplatin (100mg/m2 days 1, 22 and 43) along with concurrent radiation (2Gy per day, 5 days per week for a total of 70Gy). A fall back dose of 1000mg daily (Cohort -1) was included but not required during study. Blood draws indicated by black boxes were performed on Day -14, Day 1 and subsequently on treatment during week three and at the completion of treatment for pharmacodynamics. Pharmacokinetic blood draws were Day -14 and Day 1 at 0, 0.5, 1, 2, 4, and 24 hours. Under initial protocol, patients 1, 5, 6 and 7 were enrolled in cohort 1, and patients 2-4 in cohort 2. Under amendment, patients 8-9 were enrolled in cohort 1, 10-12 and 18-21 in cohort 2 and 13-17 in cohort 3. **C. Schematic for patient dose assignment based on mTPI design.** X-axis represents total number of patients in each cohort, y-axis represents the total number of patients with ≥ 1 DLT recorded at that dose level. The shaded area represents the first 3 patients in the

first cohort. (E) would result in escalation to the next cohort. (D) indicates de-escalation to prior dose, (S) would indicate next patient to stay at current dose and (DU) would indicate unacceptable toxicity and therefore, no patients could be escalated back to this dose for the remaining study. However, the next patient could be de-escalated to the next lower dose until no further cohorts were available. The dose most patients received is confirmed to be the MTD.

Figure 2. Kaplan-Meier curves of (A) investigator- assessed PFS and (B) OS. Hash marks indicate censored observations.

Figure 3. Metformin effects on laboratory blood levels. (A) Levels of C-peptide and (B) Vitamin B12 levels pre-metformin, during metformin and off metformin.

Table 1. Baseline Patient and Disease Characteristics. NOTE. Data presented as No. (%) unless otherwise indicated. Abbreviations: HPV, Human Papilloma Virus

Table 2. Summary of Treatment-Related Adverse Events. Abbreviations: AKI, Acute Kidney Injury; LFT, Liver Function Tests

Table 3. Pharmacokinetic Assessments of Metformin**.** Pharmacokinetic parameters of metformin for various dose cohorts computed using non-compartmental analysis. Data; mean ± SD. For analysis, serial blood samples were collected at 0, 0.5, 1, 2, 4 and 24 hrs after metformin administration on days -7 or -14 and 1. Metformin plasma levels were quantitated using a LC/MS method. Abbreviations: AUC, Area Under the Curve.

Table 1.

PATIENT CHARACTERISTICS		
Age: Median	56	
Range	(46-65yrs)	
Sex– (No., %) Male	15 (83%)	
Female	3 (17%)	
Race– (No, %) Caucasian	16 (88%)	
African American	2 (11%)	
Tobacco abusers (No., %)	11 (61%)	
p16 Positive	13 (72%)	
Primary tumor site– no. (%)		
Oropharynx	12 (67%)	
Larynx	6 (33%)	
Stage– no. (%)	AJCC 7	AJCC 8
I		4 (22%)
II		2 (11%)
III	2 (11%)	8 (45%)
IVA	12 (67%)	4 (22%)
IVB	4 (22%)	
Tumor Stage– no. (%)		
T1 or T2	4 (22%)	6 (33%)
T3 or T4	12 (67%)	10 (56%)
Tx	2 (11%)	2 (11%)
Nodal stage– no. (%)		
N0	2 (11%)	2 (11%)
N1	1 (6%)	5 (27%)
N2a or N2b	6 (33%)	5 (27%)

N2c	6 (33%)	3 (17%)
N3	3 (17%)	3 (17%)

Table 2.

ADVERSE EVENTS: CTCAE Category	**Any Grade no,%**	**Grade 1-2**	**Grade 3-4**
GASTROINTESTINAL			
Diarrhea	8 (45%)	7 (39%)	1 (6%)
Nausea	14 (77%)	12 (67%)	2 (11%)
Vomiting	9 (50%)	7 (39%)	2 (11%)
Reflux	5 (27%)	5 (27%)	0
Mucositis	5 (27%)	4 (22%)	1 (6%)
Others (abdominal pain, dysphagia, altered taste)	8 (45%)	8 (45%)	0

METABOLISM AND NUTRITION DISORDERS			
AKI	8 (45%)	5 (27%)	3 (17%)
Lactic acidosis	0	0	0
Electrolyte abnormalities	4 (22%)	4 (22%)	0
BLOOD AND LYMPHATIC SYSTEM DISORDERS			
Leucopenia/ Neutropenia	5 (27%)	3 (17%)	2 (11%)
Anemia	5 (27%)	4 (22%)	1 (6%)
INVESTIGATIONS			
LFT abnormalities	2 (11%)	2 (11%)	0
EAR AND LABYRINTH DISORDERS			
Tinnitus, hearing loss, ear pain	4 (22%)	4 (22%)	0

Table 3.

	Day -7 or Day -14	Day 1	Day -7 or Day -14	Day 1	Day -7 or Day -14	Day 1	Day -7 or Day -14	Day 1
1	500 BID	1000 BID	2.5 ± 1.2 (n = 6)	4 (n = 2)	1647 ± 551 (n = 6)	2045 ± 1022 (n = 2)	4828 ± 1469 (n = 6)	6181 ± 4340 (n = 2)
2	500 BID	850 TID	1.9 ± 0.9 (n = 9)	2.7 ± 1.2 (n = 3)	1139 ± 386 (n = 9)	2030 ± 550 (n = 3)	3572 ± 1323 (n = 9)	6354 ± 2050 (n = 3)
3	500 BID	1000 TID	2.4 ± 0.9 (n = 5)	2 (n = 3)	1215 ± 483 (n = 5)	2585 ± 1007 (n = 3)	3633 ± 1460 (n = 5)	8499 ± 3032 (n = 3)

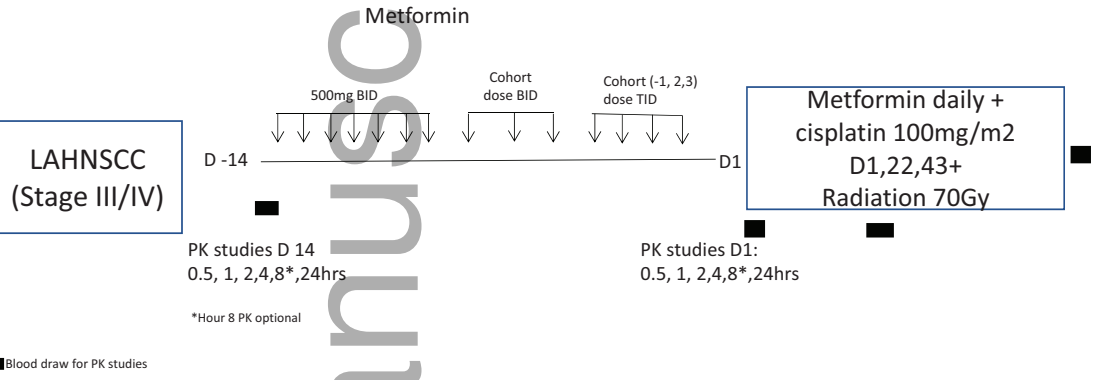
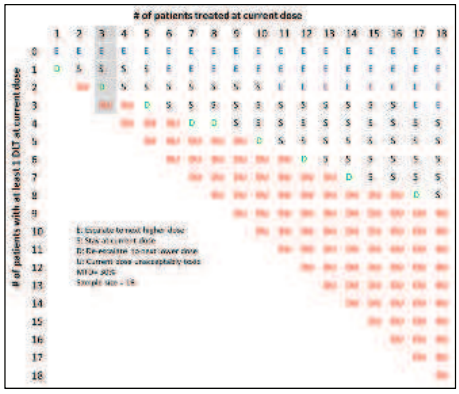


Figure 1C

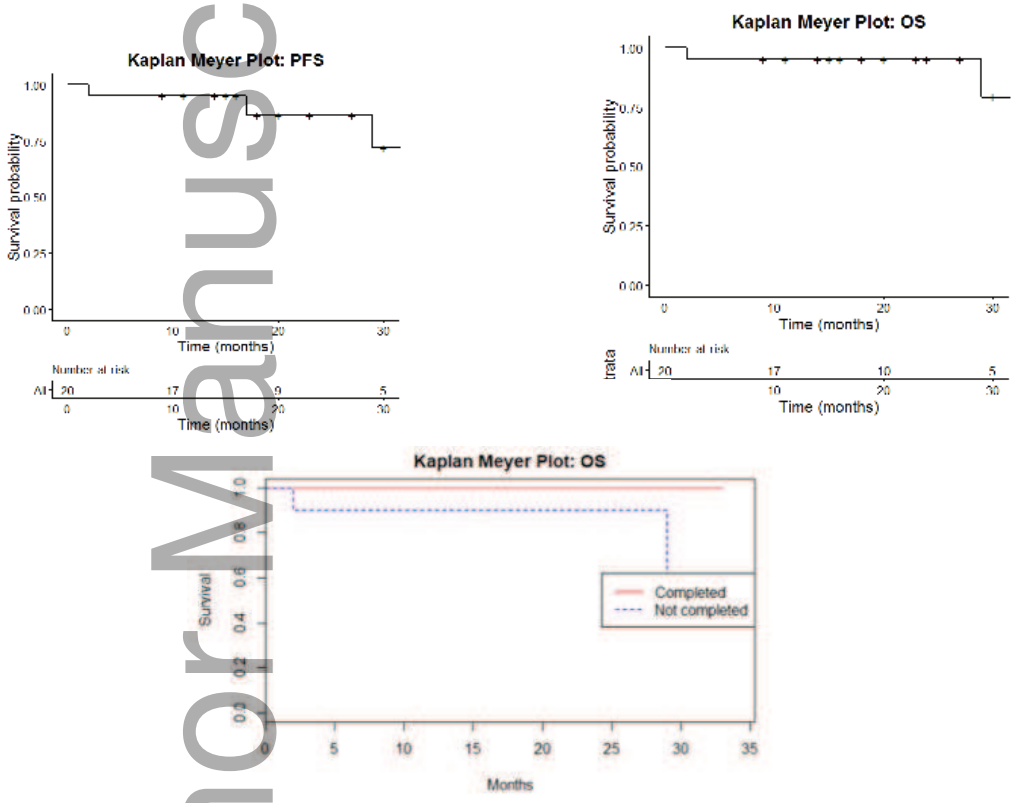


cncr_32539_f1.eps

1B

Cohort	Metformin daily (in divided doses)
-1	1500 mg (500mg TID)
1	2000 mg (1000mg BID)
2	2550 mg (850mg TID)
3	3000 mg (1000mg TID)

gure 2



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