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METFORMIN THERAPY REDUCES THE RISK OF MALIGNANCY FOLLOWING HEART TRANSPLANTATION

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ABSTRACT

BACKGROUND: Malignancy and diabetes mellitus (DM) cause significant morbidity and mortality after heart transplantation (HTx). Metformin, one of the most commonly used anti-diabetic drugs worldwide, has also been shown to exhibit anti-tumor activity. We therefore investigated the association between metformin therapy and malignancy following HTx.

METHODS: The study population comprised 237 patients who underwent HTx between 1991 and 2016 and were prospectively followed-up. Clinical data were recorded on prospectively designed forms. The primary outcome was any cancer recorded during 15 years of follow up. Treatment with metformin and the development of DM following HTx were assessed as time-dependent factors in the analyses.

RESULTS: Of the 237 study patients, 85(36%) had diabetes. Of the DM patients, 48(56%) were treated with metformin. Kaplan-Meier survival analysis showed that at 15 years following HTx, malignancy rate was 4% for DM patients treated with metformin, 62% for those who did not receive metformin, and 27% for non-DM patients (log rank *p*-value <0.0001). Consistently, multivariate analysis showed that for DM patients metformin therapy was independently associated with a significant 90% reduction (HR=0.10; 95% CI 0.02-0.40; *p*=0.001) in the risk of the development of a malignancy. DM patients who were treated with metformin experienced a markedly lower risk (65%; $p = 0.001$) for the development of a malignancy or death following HTx as compared with non-DM patients.

CONCLUSIONS: Our findings suggest that metformin therapy is independently associated with a significant reduction in the risk of malignancy following HTx.

INTRODUCTION

Heart transplantation (HTx) is the gold standard curative therapy for selected patients with end-stage heart failure. Since the first HTx in 1967,[1] survival has improved considerably. According to the latest International Society for Heart and Lung Transplantation (ISHLT) registry data,[2] median survival of adult HTx recipients is 10.7 years and 13.3 years for those surviving the first year after HTx. An important cause of morbidity and mortality after HTx is malignancy, with a rate of 5% for survivors of the first year and up to 27% in 10-year survivors (for all types combined). Perhaps even more striking statistics are those for diabetes mellitus (DM), with 23% of survivors being affected within 1 year after transplant, and 37%, within 5 years after transplant.[2] In addition, it has been suggested that DM is associated with an increased risk for the development of malignancy following HTx. [3, 4]

Metformin is one of the most commonly used anti-diabetic drugs worldwide, and exhibits anti-tumor activity both in vivo and in vitro.[5] Observational studies have shown a reduced incidence of breast,[6,7] lung,[8] and colorectal[9] cancers and of hepatocellular carcinoma (HCC)[10] in diabetic patients treated with metformin. In addition, pre-clinical studies have shown that metformin inhibits cell proliferation and tumor growth of various cancer cells, both in vitro and in vivo.[6] Moreover, the combination of metformin with chemotherapeutic drugs produced synergistic effects, permitting dose reduction and minimizing the side effects of chemotherapy.[6,11,12]

Given the high prevalence of both diabetes and malignancy in HTx patients, we designed a study to investigate the association between metformin therapy and the incidence of malignancy following HTx.

METHODS

Study population and registry

Our registry at the tertiary Heart Transplantation Clinic at the Sheba Medical Center includes 275 patients who underwent HTx and follow-up between 1991 and 2016. We excluded from the current study patients who died within 1 month of transplantation (n = 38); thus, the study population comprised 237 patients. None of the study patients had been diagnosed with malignancy prior to HTx. Data for each patient were systematically recorded upon enrollment in the study and during each subsequent visit or medical contact. Clinical data were recorded on prospectively designed forms and included comprehensive information regarding the transplantation procedure, immunosuppression, occurrence of major cardiac events, malignancy, diabetes and treatments during long-term follow-up. The study was approved by the Institutional Review Board.

Definitions and endpoints

Immunosuppression. All patients were treated with triple-drug immunosuppression based on calcineurin inhibitors (CNIs), antimetabolites, and prednisone. Since the introduction of everolimus it was applied to minority of patients later in the follow up, combined with low dose CNIs. Conversion to everolimus was considered according to patient's risk profile including cytomegalovirus infection, renal failure, allograft vasculopathy and malignancy risk.

Diabetes mellitus. DM was defined on the basis of the American Diabetes Association diagnostic criteria that were current at the time of diagnosis: hemoglobin A1c level of ≥6.5 %, or fasting plasma glucose level of ≥126 mg/dL, or random plasma glucose level ≥ 200 mg/dL. Medical management of diabetes was in accordance with known treatment recommendations, which included life-style modifications, weight control, increased physical activity, and diabetes education. Pharmacologic therapy was prescribed in accordance with the appropriate recommendations.[13] Despite significant changes in treatment guidelines for diabetes during

the past 25 years, metformin has remained the recommended tier 1 medication for diabetes management. Of note, metformin was approved and available for prescription in Israel throughout the study period.

In the present study, patients with DM were categorized into two groups according to whether or not they were treated with metformin—defined as DM-metformin and DM-non-metformin groups. There were no contraindications (metabolic acidosis, severe renal or hepatic impairment, and/or advanced congestive heart failure) for metformin treatment in any of the DM patients enrolled in the study.

Outcomes measures. The primary outcomes measures of the present study included 1) the first occurrence of a new post HTx malignancy (any type, histology confirmed) and 2) the combined end point of malignancy and all-cause mortality. Mortality data were obtained from the Population Registry of the State of Israel, which records all deaths by law. Cancer incidence date and cancer type were obtained from patients' records, a process that was verified manually and checked against pathology and imaging reports.

Statistical analysis

The clinical characteristics of the study patients were compared by treatment with metformin among DM patients, and between DM and non-DM patients, using the chi-square test for categorical variables, and the t-test for continuous normally distributed variables or non-parametric tests for those violating normality assumptions.

Malignancy (all types) and mortality outcome curves, by the presence of DM and metformin therapy, were constructed according to the Kaplan-Meier method, and curves were compared using the log rank test. In these analyses, time to event follow-up was initiated upon the date of initiation of therapy with metformin or the development of DM (among DM patients not treated with the drug). We used the formal pathology date in order to calculate time period from HTx to malignancy diagnosis.

In order to further evaluate the independent association of metformin administration and malignancy risk, we used the multivariate Cox proportional hazard model with time dependent covariates.

In the multivariate analysis, we included: 1) covariates significantly associated with outcomes in a univariate analysis; and 2) covariates that were clinically important and previously have been shown to be associated with the relevant outcomes. Age, gender, and smoking have been shown in previous studies to be important risk factors[14-16] specifically in multivariate analysis.[4] According to the ISHLT registry data male gender is associated with reduced malignancy-free survival.[2] Models were constructed according to the best subset method.

The time from HTx to metformin start or DM diagnosis was indicating a switching point in exposure status. Accordingly, pre-exposure period was set to 0 and exposure period was set to 1. The first model comprised of the DM only population for the endpoint of any malignancy during 15 years of follow-up. Covariates included in the model were the following: age, gender, metformin administration, creatinine levels, diagnosis hemoglobin A1c levels, DM pre-HTx, past smoker, and everolimus use as a time depended covariate.

To validate the consistency of the results, a secondary analysis was performed for a composite endpoint comprising the development of any malignancy or all-cause mortality during 15 years of follow-up. The model included the following covariates: age, gender, metformin administration, creatinine levels, diagnosis hemoglobin A1c levels, DM pre-HTx, past smoker status and everolimus use as a time depended covariate.

Finally, we constructed 2 additional Cox models for the outcome of malignancy, and separately for the outcome of malignancy or death, as detailed above using the entire cohort (DM and non-DM). We compared the risks associated with metformin treatment vs. diabetics without metformin, and vs. the non-DM group.

A 2-sided 0.05 significance level was used for hypothesis testing. Data were analyzed with SAS version 9.3 statistical software (SAS Institute Inc.).

RESULTS

Patient characteristics

A total of 237 patients were discharged alive after transplant, of whom 85 (36%) patients after HTx had diabetes (DM group) whereas 152 (64%) did not (non-DM group). Among the 85 DM patients, 42 (49%) had the disease prior to HTx, and 43 (51%) developed DM following HTx (n = 85). All of the DM patients developed diabetes prior to the diagnosis of a malignancy. The baseline clinical characteristics of the study patients by the presence of DM and by therapy with metformin for DM patients are given in Table 1. Of the DM patients, 48 (56.5%) were treated with metformin (DM metformin group) and 37 (43.5%) patients were not (DM non-metformin group). Baseline immunosuppressive protocol did not differ between groups, the minority of patients were treated de novo with everolimus (Table 1), however 37 patients overall were treated with everolimus later during the follow-up.

Characteristics of DM vs. non-DM patients. HTx patients with DM were approximately 7 years older than the non-DM patients, and their donors were approximately 4 years older. A comparison of the two groups prior to HTx revealed that the DM patients had a higher frequency of ischemic heart disease as etiology for HTx, higher rates of hypertension and dyslipidemia, and higher systolic and diastolic pulmonary artery pressures (Table 1). At the time of transplant, weight and height were higher in DM patients than in the non-DM patients (Table 1).

Characteristics of DM patients by treatment with metformin. There were no statistically significant differences between patients with DM who were or were not treated with metformin in terms of the donor's or the recipient's age, gender, underlying pre-transplant cause of heart failure, pre-existing cardiovascular risk factors and hemodynamics before HTx (Table 1). Ischemic time was longer and there was a trend toward a higher frequency of hypertension after HTx in the metformin-treated group. In addition, in the DM non-metformin group there was a somewhat higher frequency of patients who developed DM

following HTx than in the DM-metformin group (Table 1). The mean dose of metformin use was 1097.9 (± 428) mg per patient. The main reasons for avoiding metformin treatment were kidney dysfunction; patient concern of common side effects, (mainly gastrointestinal adverse effects because of similar side effects associated with immunosuppressive regimens); presence of risk factors associated with lactic acidosis in patients who do not necessarily currently have specific contraindications to its use.

Clinical outcomes

Risk of malignancy.

Kaplan-Meier estimates of the end point of malignancy for all patients are shown in Figure 1A, demonstrating that the rate of malignancy was lowest for DM patients treated with metformin, intermediate for non-DM patients, and highest for DM patients who were not treated with metformin. Thus, at 15 years of follow-up, the respective rates of malignancy in the 3 groups were 4%, 27%, and 62% (log-rank p-value < 0.001 for the comparison among the 3 groups during follow-up; Figure 1A).

Consistent with these findings, multivariate analysis showed that for DM patients treatment with metformin was associated with a significant 90% (p = 0.001) reduction in the risk for the development of any malignancy following HTx (Table 2A – left panel). Notably, overall, the risk of malignancy was not significantly higher for DM than for non-DM patients [hazard ratio (HR) = 0.911; p = 0.66; Table 2B]. Increased creatinine and age were independently associated with higher risk of malignancy in the entire cohort (Table 2B).

Risk of the composite endpoint of malignancy or all-cause mortality.

Kaplan-Meier estimates of combined end point of malignancy or death for all patients are shown in Figure 1B. The rate of combined end point was significantly lowest for DM patients treated with metformin, intermediate for non-DM patients, and highest for DM patients who

were not treated with metformin. Thus, at 15 years of follow-up, the respective rates of combined end point in the 3 groups were 45%, 58%, 83% (log-rank *p*-value = 0.0002 for the comparison among the 3 groups during follow-up; Figure 1B).

Consistent with these findings, multivariate analysis showed that for DM patients treatment with metformin was associated with a significant 79% ($p < 0.001$) reduction in the risk for combined end point following HTx compared with diabetic patients without metformin treatment (Table 2A- right panel). Notably, overall, the risk of combined end point was not significantly different for DM and non-DM patients (HR 0.89; $p = 0.621$; Table 2B-right panel). However, for the DM patients treated with metformin there was a significantly 65% reduction in the risk of malignancy or death as compared with non-DM patients (HR=0.35; P=0.001). Past smoking and diagnosis of DM pre HTx were independently associated with increased risk of malignancy or all-cause mortality in the entire study cohort and in the DM only group.

Malignancy characteristics

The number of events per 100 person-years (namely, the event rate) of any cancer was 1.03 in the DM metformin group, as compared to 6.48 for the DM non-metformin group and 2.48 for the non-DM group ($p = 0.012$; Table 3). For the three groups, the event rates for skin cancers were 0.21, 4.44, 1.24, respectively ($p = 0.004$; Table 3).

Malignancy Type

The distribution of malignancy type according to the presence/absence of DM is shown in Figure 2. Skin cancers constituted the most common type of malignancy (50%) in both non-diabetic and diabetic patients.

DISCUSSION

Diabetes and malignancy are major confounders of mortality and morbidity after HTx, and every effort should therefore be made to reduce their burden. To the best of our knowledge, the present study is the first to address the impact of metformin treatment on outcomes in diabetic patients after HTx. Our findings have several important implications for the management of patients undergoing HTx. We have shown that: 1) for DM patients, treatment with metformin is associated with a significant and pronounced (90%) reduction in the risk for the development of malignancy following HTx; and 2) patients with DM who were treated with metformin exhibited a significantly lower risk of developing a malignancy or death following HTx compared with non-DM patients. These findings suggest a possible role for metformin therapy in all patients who undergo HTx.

Diabetes after HTx

Diabetes is common in heart transplant recipients and is associated with increased rates of infection, cardiac allograft vasculopathy (CAV), graft loss, and reduced survival. [2,17-21] Risk factors for the development of new onset DM after transplant are similar to those in the general population, yet the immunosuppressive treatments have additional contribution to the development of diabetes: Such treatments include corticosteroids, known for their profound effects on carbohydrate metabolism, CNIs, which impair insulin secretion and sensitivity, inhibit insulin gene transcription, and cause direct damage to pancreatic islet cells,[22,23] and mTOR inhibitors, which have been shown to exhibit islet cell toxicity.[24,25]

In our cohort, 36% of patients had diabetes after HTx. It has previously been shown that diabetes is a risk factor for survival after HTx,[21] but post transplantation survival among patients with uncomplicated DM is not significantly different from survival of non-diabetics.[26] We believe that treatment with metformin would further blur these differences. In our study, we showed that the combined end point of malignancy or death did not differ between the diabetes and non-diabetes groups. Metformin was a possible contributing factor, as sub group analysis showed that in diabetic patients treated with metformin there was a

significantly reduced (65%) risk for the combined end point compared to the non-diabetic patients. As a previous study has suggested an inverse relationship between total metformin's dose and duration of exposure, and the risk of cancer[27], we suggest that metformin administration should be considered as early as possible, even in diabetic patients being evaluated for HTx.

Metformin treatment for diabetes after HTx

Management of post-transplant diabetes should generally conform to the guidelines for treatment of DM in the general population.[21] Metformin is generally the initial oral agent of choice for patients with type 2 DM, yet there is a paucity of data related to metformin use post transplantation. This lack of data is surprising in light of metformin's potential benefits, among which are antiglycemic efficacy, attenuation of metabolic syndrome, cardiovascular protection, lipid-lowering benefits, neutral weight maintenance or potential weight reduction, and anti-neoplastic potential.[28] Furthermore, metformin is not metabolized by CYP3A4 and therefore has no drug-drug interactions with immunosuppressive medication.

Metformin is cleared renally, and concerns of lactic acidosis in such settings have been established. [29] The Food and Drug Administration recommends that metformin should not be used in men with a serum creatinine level of ≥1.5 mg/dl or women with a serum creatinine level of ≥1.4 mg/dl. These recommendations obviously impinge on its use in HTx patients, approximately half of whom display some level of renal dysfunction at 5 years post-transplant, yet more than half of them have creatinine levels < 2.5 mg/dl.[2] Studies of patients with glomerular filtration rates (GFRs) in the range of 30–60 ml/min/1.73 m^2, who continue to receive metformin, demonstrate that lactic acidosis is exceedingly rare, [29,30] thus possibly permitting the use of metformin in the majority of diabetic patients after HTx.

Metformin for malignancy

In the past decade, evidence supporting metformin's potential activity against many different types of cancer has accumulated. The potential broad antineoplastic effects of metformin, alone or combined with standard chemotherapeutic agents, have been observed in various cancers, with metformin's mode of action being attributed to the inhibition of cell proliferation, tumor growth, protein synthesis, angiogenesis, and metastasis, to targeting cancer stem cells, and/or to reversing multidrug resistance. [27,31-34] Oral co-administration of metformin with multiple chemotherapeutic agents has been found effective against a variety of cancer cell types, and there are also reports of metformin facilitating both a reduction in chemotherapy dose, leading to lower toxicity, and also prolonged remission. In our study, a significantly lower risk of malignancy was observed in diabetic patients treated with metformin. The reduction in cancer incidence is not attributed to shorter survival, as metformin also improved cancer-free survival.

In 2010, a consensus statement issued by the American Diabetes Association and the American Cancer Society concluded that "although still limited, early evidence suggests that metformin is associated with a lower risk of cancer."[35] In the current study, we found that metformin treatment of HTx patients was associated with a significantly reduced risk of cancers of all types (Figure 2), of skin cancers (Table 3), and of the combined end-point of cancer or death (Figure 1). In our study, the risk reduction for malignancy was higher as compared to values reported by meta-analyses, which suggested that metformin is cancer incidence by 30-50%.[36] The variation in the effects of metformin could be explained by differences in both patient characteristics and tumor biology. Lee et al. have demonstrated that simultaneously blocking glycolysis and glutamine pathways can effectively inhibit allo-specific T cell responses while preserving mechanisms of immune regulation. [37] Such a mechanism has therapeutic potential through prevention of graft rejection, as well as the maintenance of cellular regulation, thereby preventing cell transformation.

Mechanisms of metformin as an anti-cancer drug and implications for immunosuppression

The fundamental mode of action of the drug is to reduce mitochondrial oxidative phosphorylation, thereby inducing energy stress. Metformin-induced energetic stress inhibits hepatic gluconeogenesis, leading to lower circulating glucose levels and a fall in insulin levels. This can have a cytostatic effect on the subset of tumours that thrive in a high-insulin environment[38] and can potentially counteract the Warburg effect (dependence of cancer cells on glucose as the predominant source of energy).[39,40] The cellular and direct effects are believed to involve activation of the AMPK pathway,[41-43]by reducing energy-consuming biosynthetic functions, such as protein synthesis (via AMPK-dependent mTOR inhibition) and lipid synthesis (via AMPK-dependent FAS inhibition), eventually leading to inhibition of protein synthesis and reductions in cell growth and proliferation. Certain tumours have defects that render them incapable of compensating for the energetic stress induced by metformin, leading to a cytotoxic effect on the tumour, with no important effect on the host, and a favourable therapeutic index.[38]

The mammalian target of rapamycin (mTOR) inhibitors, introduced for the treatment of organ transplantation, confirm an antitumor potential and safer malignancy profile.[44] Given the above mechanism and involvement of the AMPK-dependent mTOR inhibition, the hypothesis of synergist effect with newer immunosuppressive as mTOR inhibitors, should be further explored. The use of mTOR inhibitors did not significantly influence outcomes in our multivariate analysis, nevertheless the relatively small number of patients and late introduction of this therapy needs to be further explored in larger studies.

Preemptive metformin treatment

There is evidence to suggest that metformin treatment in non-diabetic patients would support complete response in females with breast cancer.[45] The combination of a high rate of malignancies after HTx, metformin's safety profile, paucity of interactions with other drugs,

and the results of the present study, raises the question of the prophylactic use of metformin for the prevention of cancer after HTx. Future studies should evaluate the possibility, suggested by our present findings, that the observed reduced malignancy rate with metformin following HTx may extend beyond better diabetic control, to a possible anti-tumor effect which may benefit even non-diabetics.

Study limitations

The major limitation of our study is its retrospective nature. Because tumor genesis is a slow process, a sustained exposure to medication is needed in order to prove causality, a short period of treatment in some patients argues against a causal relationship. Furthermore, not all possible confounders were recorded or adjusted for in this single-center study. We should also point out that the confidence intervals were wide, indicating that the sample size was too small to draw definitive conclusions; thus, any conclusions drawn from the data must be replicated with a larger sample size and a prospective multicenter design.

Conclusions and clinical implications

The results of the present study suggest that metformin therapy may be associated with a reduced risk for the development of malignancy following HTx. The role of metformin therapy as an anticancer agent in patients undergoing HTx must be further evaluated in prospective clinical trials. Nonetheless, at present, our findings suggest that all HTx patients with DM should receive metformin therapy, unless there are contraindications for its administration.

Disclosure statement

None of the authors has a financial relationship with a commercial entity that has an interest in the subject of the presented manuscript or other conflicts of interest to disclose.

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Figure Legends

Figure 1: Kaplan-Meier estimates for malignancy rates (A) and for the outcome of malignancy or death (B) in the entire study population during 15 years of follow-up. Population is grouped by DM status and in the DM group by metformin treatment or lack of metformin use.

Figure 2: Type of malignancy by diabetes mellitus status as obtained from the pathology reports. All cancer types were included. A number of subjects presented with more than one type of cancer during the follow-up period.

Table 1: Baseline characteristics of the Patients by Diabetes Status and the Use or Non-Use of Metformin in the Diabetes Group

	DM ***N*=85**		**Non-DM** ***N*=152**
	Metformin Rx ***N*=48**	**Non-Metformin Rx** ***N*=37**	
Before heart transplantation			
Recipient age (years)	52±9	54±9	46±15†
Donor age (years)	34±12.6	37±11.9	31±12
Etiology– ischemic	71	78	49†
Recipient gender (female)	14.6	10.8	20.4
Donor gender (male)	74	72	74
Recipient weight (Kg)	80±14	78±11	70±17†
Recipient height (cm)	174±7	171±9	167±23†
Hypertension	60	60	24†
Past Smoker	56	51	36
Recipient Blood Type			
A	44	39	46
AB	20	6	9
B	16	16	22
O	20	39	23
Place of HTx - Israel	72	72	65
Dyslipidemia	77	65	36†
Recipient bilirubin (mg/dl)	1.79±3	1.05±0.5	1.04±0.6
Recipient creatinine (mg/dl)	1.3±0.09	1.6±0.3	1.2±0.5
Inotropic	64	65	66
PASP (mmHg)	57±20	50±14	48±18†
PADP (mm Hg)	29±10	24±10	23±10†
Mean PAP (mm Hg)	38±13	36±12	33±13

LVAD bridge to HT	15	11	10
Ischemic time (min)	165±35*	142±35*	165±53
After heart transplantation			
Newly diagnosed DM	42	62	NA
HbA1c% at diagnosis	8.4±1.3*	7.3±0.7*	NA
Creatinine† (mg/dl)	1.08±0.2*	1.47±0.7*	
Statin treatment	98	95	92
Hypertension	100	92	68†
Insulin treatment	62	65	NA
Aspirin treatment	46	41	38
Immunosuppression			
Cyclosporine based	69	76	60
Tacrolimus based	31	23	35
Everolimus based	0	3	5
Follow up (years)	9(5-13)	8(5-12)	8 (3-14)

Continuous variables and categorical variables are presented as mean±standard deviation and percentage, respectively.

The years of follow up are presented as median values (interquartile range).

*p<0.05 for the comparison of DM-metformin versus DM non-metformin

†p<0.05 for the comparison of DM versus Non-DM

† Creatinine at diagnosis of DM or metformin initiation

DM- diabetes mellitus, Rx- treatment, HTx – heart transplantation, PASP- pulmonary artery systolic pressure, PADP- pulmonary artery diastolic pressure, PAP- pulmonary artery pressure, LVAD– left ventricular assist device, NA- not applicable.

Table 2: Multivariate Cox Proportional Hazard Models with Time Dependent Covariate Analysis for Malignancy and Composite of Malignancy or Death Outcomes During 15 Years of Follow-Up

Table 2A: DM Only Group – Metformin vs. Non-Metformin *

	Malignancy		Malignancy or death	
Risk Factor	**HR** **95% CI**	***P* Value**	**HR** **95% CI**	***P* Value**
DM metformin vs. DM non-metformin	0.10 0.02-0.40	0.001	0.21 0.10-0.44	0.0001
Age (per 1 year increment)	1.04 0.97 -1.12	0.288	1.01 0.99-1.03	0.129
Gender	1.05 0.95 -1.52	0.378	1.07 0.99-1.15	0.217
DM diagnosis Pre-HTx	1.98 0.65-6.12	0.23	2.13 1.10-4.12	0.024
Creatinine (per 1 unit increment)	1.45 0.97-2.18	0.07	1.35 1.10-1.82	0.044
Past smoker vs. non-smoker	1.15 0.40-3.43	0.79	2.24 1.21-3.28	0.001
mTOR inhibitors (as time dependent covariate)	0.88 0.41-3.42	0.69	0.64 0.33-4.13	0. 51

HR- Hazard ratio, CI- 95% Confidence Interval, DM-Diabetes Mellitus, HTx- Heart Transplantation, mTOR-mammalian target of rapamycin

* Models further adjusted Hb A1c % (p-value>0.1)

Table 2B – Entire Study Cohort *

	Malignancy		Malignancy or death	
Risk Factor	**HR** **95% CI**	***P* Value**	**HR** **95% CI**	***P* Value**
Overall: DM vs. non-DM	0.911 0.33-1.91	0.66	0.89 0.55-1.71	0.621
DM metformin vs. non-DM	0.58 0.26-1.32	0.196	0.35 0.16-0.73	0.001
DM non-metformin vs. non-DM	1.71 0.76-3.86	0.19	1.38 0.79-2.42	0.37
Age (per 1 year increment)	1.07 1.03 -1.12	0.001	0.98 0.99-1.02	0.526
DM diagnosis Pre-HTx	1.73 0.69-3.12	0.312	2.21 1.10-4.45	0.025
Gender	1.33 0.60-4.95	0.43	2.44 0.77-2.44	0.614
Creatinine (per 1 unit increment)	1.48 1.01-2.18	0.044	1.25 0.99-1.57	0.059
Past smoker vs. non-smoker	2.00 0.98-4.07	0.058	1.88 1.21-2.91	0.004
mTOR inhibitors (as time dependent covariate)	0.98 0.41-3.11	0.69	0.84 0.37-6.73	0. 66

HR- Hazard ratio, CI- 95% Confidence Interval, DM-Diabetes Mellitus, HTx- Heart Transplantation, mTOR-mammalian target of rapamycin

Table 3: Incidence Rate of Various Events by 100 Person-Years*

	DM						**Non DM**			
	Metformin Treatment			**Non Metformin Treatment**						
	N events	Person Years	Event Rate	*N* events	Person Years	Event Rate	*N* events	Person Years	Event Rate	*p* value
Mortality	18	463	3.89	22	331	6.65	59	1374	4.29	0.065
CAV	14	443	3.16	9	319	2.82	34	1251	2.72	0.727
Skin Cancer	1	484	0.21	13	293	4.44	16	1288	1.24	0.004
Any Malignancy	5	484	1.03	19	293	6.48	32	1288	2.48	0.012

*p value was calculated using negative binominal distribution

Figure 1A: Kaplan-Meier Curves for Malignancy: all patients

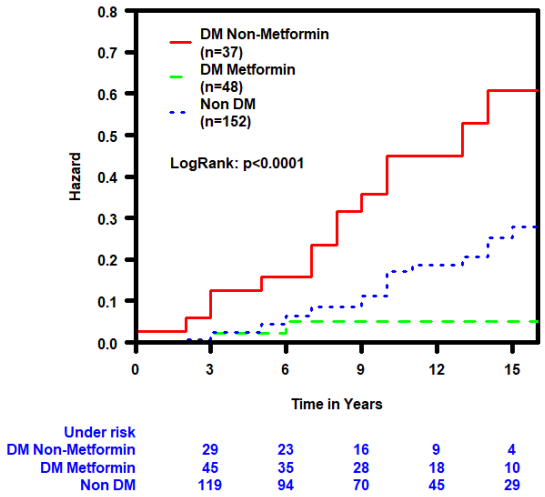

Figure 1B: Kaplan-Meier Curves for Combined End Point of Malignancy or Death: all patients

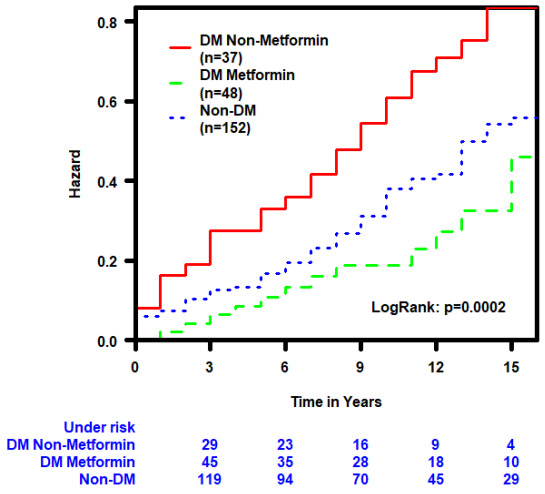

Figure 2: Type of Malignancy by Diabetes Mellitus Status