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# Additive interaction between renin-angiotensin system and lipid metabolism for cancer in type 2 diabetes mellitus

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*Objective*– Clinical and experimental studies suggest cross-talk between lipid metabolism and the renin-angiotensin system (RAS) in atherogenesis. This study aimed to explore interactions between these two systems in mediating cancer risk in Type 2 diabetes mellitus (T2DM).

*Research design and methods* - A prospective cohort of 4160 Chinese patients with T2DM, free of cancer at enrolment, was analyzed using Cox models. Interaction of RAS inhibitors (angiotensin I converting enzyme inhibitors or angiotensin II receptor blockers) and statins was estimated using relative excess risk due to interaction (RERI), attributable proportion due to interaction (AP) and synergy index (S). RERI >0, AP >0 or S >1 indicates additive interaction between the two classes of drugs. Molecular mechanisms underlying these interactions were explored using a uninephrectomy (UNX) rat model with renal carcinogenesis.

*Results* - During 21,992 person-years of follow-up, 190 patients developed cancer. Use of RAS inhibitors and statins during follow-up was associated with reduced risk of cancer after adjusting for covariates. The multivariable RERI and AP for the additive interaction between these drug classes in cancer patients were significant (0.53, 95% confidence interval: 0.18 to 0.86; and 2.37, 95%CI: 0.27 to 4.48, respectively). In the UNX rat model, inhibition of the RAS prevented renal cell carcinoma by normalizing HMG-CoA reductase (HMGCR) expression and insulin-like growth factor-1(IGF-1) signaling pathway.

*Conclusion* - Combined use of RAS inhibitors and statins may act synergistically to reduce cancer risk, possibly, via HMGCR and IGF-1 signaling pathways in high risk conditions such as T2DM.

Type 2 diabetes mellitus (T2DM) is associated with increased risk of a variety of cancers(1) such as colorectal (2), pancreatic (3) and liver cancers (4), as well as breast (5) and endometrial cancers (6) in women and prostate cancers in men (7). In Hong Kong, type 2 diabetic patients have a 30% increased risk of cancer compared with the general population (8). We have previously reported non-linear relationships between lipids and cancer risks in T2DM (9). The risk association of cancer with low-density lipoprotein cholesterol (LDL-C) was V-shaped, with both LDL-C levels of < 2.80 mmol/L and ≥ 3.80 mmol/L being associated with elevated risks of cancer (8).

Large scale epidemiological studies have suggested that the use of renin-angiotensin system (RAS) inhibitors is associated with reduced risk of new onset of cancer (10-12), but whether statin use alters cancer risks remains controversial (13; 14). Based on our previous findings on the non-linear relationships between lipids and cancer risk in T2DM (8; 9), we hypothesized that the mevalonate pathway, which leads to cholesterol synthesis, can produce other molecules such as isoprenoids farnesol and geranylgeraniol and these small proteins are involved in cell proliferation, differentiation, and apoptosis (8). There is now consistent data from experimental, animal and human studies suggesting the activation of local/systemic RAS in T2DM (15). In a retrospective survey, T2DM patients treated with ACE inhibitors were found to have a lower risk of cancer compared to those not receiving this drug (16). In support of interactions between dyslipidaemia and RAS activation in atherogenesis (17), the combined use of rosuvastatin, a HMG-CoA reductase (HMGCR) inhibitor, and candesartan, an angiotensin receptor blocker (ARB), has been shown to have synergistic effects in reducing atherosclerosis in animal studies (18).

Amongst different growth promoting pathways, there is emerging evidence suggesting that components of the insulin-like growth factor-1 (IGF-1) system may be implicated in atherogenesis, T2DM and cancers. Binding of IGF-1 and insulin to their receptors results in activation of the PI3-K/Akt signalling pathway and protein kinase C (PKC) ζ. IGF-1 and insulin signalling systems have important roles in energy metabolism and cell growth associated with diabetes risk and cancer (19). Interestingly, inhibition of HMGCR activity by statins caused growth arrest via depressing the expression of functional IGF-1 receptor in multiple cancer cells (20), and thus statins could have therapeutic significance in IGF-1 dependent neoplasms (21; 22). In addition, RAS inhibition attenuates IGF-1 induced cardiac fibroblast proliferation (23) and elevates IGFBP3 levels among hypertensive older adults (24).

It has long been recognized that nephrectomy in rats (25) and humans (26) leads to compensated remnant kidney growth, proteinuria and hypertension, and is associated with local RAS activation. Recently, we reported the presence of elevated blood glucose and blood lipids, associated with chronic renal impairment and insulin resistance in uninephrectomized rats followed-up for 10-months after operation (27; 28). These findings suggest that the uninephrectomized rat model may serve as a useful model for the study of metabolic disorders and complications related to T2DM; including the possible interaction between RAS activation and lipid metabolism for cancer in T2DM.

Against this background, we hypothesized that combined use of RAS inhibitors and statins is associated with

reduced cancer risk in T2DM and that these clinical benefits may be mediated via modulation of the HMGCR and IGF-1 pathways.

## RESEARCH DESIGN AND METHODS

**Epidemiological analysis: *Patients*-** Details on the methodology of the cohort study have been described previously (8). The Hong Kong Diabetes Registry was established in 1995 and enrolls 30-50 ambulatory diabetic patients per week. Patients were referred by general practitioners and internists from community- and hospital-based clinics, or were discharged from the Prince of Wales Hospital or other regional hospitals. Less than 10% of these enrolled patients have had hospital admissions within 6-8 weeks prior to assessment.

The 4-hour assessment of complications and risk factors was performed on an outpatient basis, and modified from the European DiabCare protocol (29). Once a diabetic subject had undergone this comprehensive assessment, he/she was considered to have entered this study cohort and would be observed until death. Ethical approval was obtained from the Clinical Research Ethics Committee of the Chinese University of Hong Kong.

Hong Kong has a highly subsidized health care system. The Hospital Authority is the governing body of all public-funded hospitals and outpatient clinics and provides 95% of the total hospital bed-days and 80% of the outpatient visits (30). All patients attending Hospital Authority hospital clinics either as outpatients or inpatients are dispensed medications on site. Clinical endpoints, including discharge diagnoses of hospital admissions and mortality from 1 January 1995 until 30$^{th}$ July 2005 were used for defining the endpoints. The Hospital Authority Central Computer System was used to retrieve all hospital admissions and drug-dispensing data. These databases were successfully matched by a unique identification number, the Hong Kong Identity Card number, which is compulsory for all residents in Hong Kong, and used by all government departments and major organizations.

From 1995 to 2005, 7920 diabetic patients were enrolled in this registry. We limited the analysis to 7387 patients who were enrolled after 1$^{st}$ December 1996 when dispensing data were computerised. The following exclusion criteria were applied prior to analysis: diagnosis of type 1 diabetes mellitus (n=323) (31), missing data on types of diabetes (n=5), non-Chinese or unknown nationality (n=45), cancer or on cancer treatment at enrolment (n=175), missing values for variables used in the analysis (n=736, See table 2 foot notes for the variable list). LDL-C is a major confounding factor for cancer in T2DM (8). Since the pre-treatment LDL-C levels were not documented in the registry and use of statins and RAS inhibitors may modify risk associations between LDL-C and cancer, we excluded 827 patients who were using statins and 1116 patients who were using RAS inhibitors at enrolment in order to reduce confounding effects due to treatment at baseline. A total of 4160 patients were entered into the present analysis (Figure 1).

**Definition of endpoints:** All hospital discharge principal diagnoses including cancer and non-cancer-related hospital admissions were regularly coded by a team of trained personnel under the Hospital Authority, according to the International Classification of Diseases, Ninth Revision (ICD-9). Mortality data from the Hong Kong Death Registry was retrieved and the causes of death were verified against hospital admission records in the Hong Kong Hospital Authority Computer System. ICD-9 codes were used to identify first admissions relating to a diagnosis of cancer. The endpoint of this

study was defined as having a first cancer event during the follow-up period (code 140-208), including fatal and nonfatal cancer.

**Clinical and laboratory measurements:** On the day of enrolment, all patients attended the Centre after at least 8 hours of fasting, and without taking any medication. A sterile, random spot urine sample was used to measure albumin to creatinine ratio (ACR). Albuminuria was defined as ACR ≥ 2.5 mg/mmol in men and ≥ 3.5 mg/mmol in women. Total cholesterol, triglyceride and high-density lipoprotein cholesterol (HDL-C) were measured by enzymatic methods on a Hitachi 911 automated analyzer (Boehringer Mannheim, Mannheim, Germany) using reagent kits supplied by the manufacturer of the analyzer. LDL-C was calculated by the Friedewald's equation (32). The precision performance of these assays was within the manufacturer's specifications.

**Statistical analyses:** The Statistical Analysis System (SAS, Release 9.10) was used to perform all statistical analysis (SAS Institute Inc., Cary, North Carolina, USA). Cox proportional hazard regression was used to obtain hazard ratios with 95% confidence intervals. Follow-up time was calculated as the period from enrolment to the date of first admission for cancer, death or 30$^{th}$ July 2005, whichever came first.

We tested multiplicative and additive interactions between use of RAS inhibitors and statins for cancer. Multiplicative interaction was tested using a term of the product of two variables in Cox models. There are three measures to test additive interaction (33; 34): 1) Relative excess risk due to interaction (RERI); 2) Attributable proportion due to interaction (AP); and 3) Synergy index (S). The RERI is the excess risk due to interaction relative to the risk without exposure. AP refers to the attributable proportion of disease which is due to interaction among persons with both exposures. S is the excess risk from both exposures when there is an additive interaction, relative to the risk from both exposures without interaction. PERI > 0, AP > 0 or S > 1 indicates additive interaction. In Cox models, the RERI is the best choice among the three measures (35). A detailed calculation method of additive interaction including definition of three indicator variables, a SAS program and a calculator in Excel (available at: www.epinet.se) was described by Andersson et al (34). Briefly, the three indicator variables were generated for different combinations of exposure to use of statins and use of RAS inhibitors (1=Yes/0=No) (See additive interaction models of table 2 for details). The SAS program delivered estimates of the required parameters together with the covariance matrix, which are used in calculation of the interaction measures in the Excel calculator.

To control for confounding effects of drug use, we used Yes/No coding which was a more robust measure than the duration of drug use with or without adjustment for the period of discontinuation (8). A structured adjustment scheme was used to evaluate the additive interaction of the use of statins and that of RAS inhibitors. Firstly, we adjusted for LDL-C related risk factors, i.e., LDL-C ≥3.80 mmol/L and LDL-C <2.80 mmol/L plus albuminuria (unpublished data), age, sex, body mass index (BMI), and the use of tobacco and alcohol. Secondly, we further adjusted for metabolic variables (See table notes of table 2 for details) and drug use from enrolment to cancer, death or censoring dates. To avoid over-fitting, a propensity score was used to adjust for the covariates, in which restricted cubic spline with 4 knots at 5$^{th}$, 35$^{th}$, 65$^{th}$ and 95$^{th}$ percentiles was used to adjust for confounding effects of non-linear associations of lipids and other continuous covariates as before (8). Stratified Cox models on deciles

of the propensity score were used in all the Cox models to adjust for likelihoods of drug use during follow-up or cancer where appropriate (36) (See table 2 for details). Proportional hazard assumptions of baseline variables and correlations between pairs of baseline variables were also checked as previously (8). A two-sided P value < .05 was considered to be significant.

**Animal experiments:** ***Animals-*** We have developed a uninephrectomy (UNX) rat model characterized by renal carcinogenesis, RAS activation and dysmetabolism of glucose and lipids to examine disease mechanisms and drug effects. Details of the experimental protocol and phenotypes have been described (27; 28). Male Sprague-Dawley rats (300-350 g) were obtained from the Laboratory Animal Services Centre at the Chinese University of Hong Kong and maintained at our Research Unit at the Prince of Wales Hospital. The animals were caged in pairs, housed at 22 ~24°C with a 12-hour dark/light cycle with free access to water, and fed on a standard laboratory rat diet (5001 Rodent Diet; LabDiet, St. Louis, MO, USA). The total duration of the studies was 10 months.

The animals were randomized into 3 groups: sham operation (n=8), left uninephrectomy (UNX, n=8), and UNX rats treated with the angiotensin converting enzyme inhibitor (ACEI)-lisinopril (ACEI, n=8). Lisinopril were dissolved in 3 ml sterile distilled water, with once daily dosage of 4 mg/kg body weight. All the sham and UNX rats were also gavaged with distilled water (3 ml) as placebo control.

Ethical approval for the animal study was obtained from the Animal Experimentation Ethics Committee of The Chinese University of Hong Kong, and in accordance to the Animals (Control of Experiments) Ordinance of the Department of Health of the Hong Kong SAR Government.

**Biochemical studies:** At 3, 6, 8 months post-operation, 24-hour urine samples were collected using metabolic cages (Huang Qiao Yin Xing Animal Cage & Equipments Factory, Suzhou, China). When rats were sacrificed 10-months post operation, fasting blood samples were taken for the measurement of renal function and lipids including total cholesterol, triglyceride, LDL-C and HDL-C.

**Histological studies of kidneys:** Histopathological criteria for diagnosis of renal cell carcinoma in the UNX rats included cytologic atypia, bizarre nuclei, frequent mitotic figures, and invasive growth. Absence of these morphological characteristics in at least three tissue sections indicated absence of renal cancer. Rats were sacrificed at 10 months post-operation. Kidneys from all the rats were removed, weighted and processed for light microscopy. Tissue samples were fixed in 10% neutral formaldehyde and embedded in paraffin. Serial longitudinal sections (4 μm) were spliced parallel to the longest axis of the kidney and stained with periodic acid-Schiff (PAS). Stained slides were examined with a Zeiss Axioplan 2 imaging microscope (Carl Zeiss, Inc, Hamburg, Germany) and representative images were captured using a Spot digital camera.

**Western blot assays:** Tissue total proteins from renal cortex were extracted. The resolved proteins were then transferred onto nitrocellulose membranes. The membranes were blocked for 1 hour at room temperature with 5% skimmed milk, incubated with primary antibodies against IGFBP3 (dilution 1:1,000; Santa Cruz Biotechnology, CA), Akt1/2/3 (dilution 1:1,000; Cell Signaling Technology, MA), and PKCζ (dilution 1:1,000; Santa Cruz Biotechnology) in TBS containing 0.05% Tween 20 (TBS-T) with 5% skimmed milk overnight at 4°C. After washing with TBS-T, membranes were

incubated with anti-goat, anti-rabbit or anti-mouse secondary antibody conjugated to horseradish peroxidase (Upstate, Temecula, MA) with dilution of 1:2,000. Proteins were detected by enhanced chemiluminescence (Amersham, Piscataway, NJ) on Hyperfilm. The major protein bands with approximately 42 kD for IGFBP3, 60 KD for Akt1/2/3, and 80 kD for PKC ζ were detected. To ensure equal loading of proteins, membranes were incubated and probed with a rabbit anti-β-actin antibody (Abcam, Cambridge, MA) with dilution of 1:10,000, which recognizes β-actin at approximately 43 kD. Signals were quantitated by densitometry and corrected for the β-actin signal, using the Kodak Digital Image station 440CF and the ID Image Analysis software program. Treatment groups were compared using analysis of variance and a $p<0.05$ was considered statistically significant.

## RESULTS

**Characteristics of the patients:** The median age of the cohort was 55 (the 25$^{th}$ to 75$^{th}$ percentiles: 45 to 66) years, with a median duration of diabetes of 5 (1 to 10) years. During a total of 21,992 person-years of follow-up, 190 patients developed cancer giving an annual incidence of 8.64 (95% CI: 7.42 to 9.86) per 1000 person-years. Patients who subsequently developed cancer were older, more likely to be smokers and alcohol drinkers and had longer duration of diabetes and poorer metabolic profile than those without (Table 1). They were also more likely to use antihypertensive drugs other than RAS inhibitors than patients without. Patients who developed cancer were less likely to be treated with statins alone or combined with RAS inhibitors during follow up period than those who remained free of cancer.

**Additive interaction between statins and RAS inhibitors:** Compared to non-users of statins and RAS inhibitors, subjects who were exposed to statins and/or RAS inhibitors had lower risk of cancer after adjusting for drug use indications and demographic and life style covariates. The additive interaction between statins and RAS inhibitors on cancer risk was significant as indicated by the RERI (0.39; 95% CI: 0.09 to 0.69) and AP values (1.57: 95% CI: 0.21 to 2.94). The statistical significances of use of statins only, use of RAS inhibitors only, combined use of statins and RAS inhibitors, and additive interaction measures (RERI and AP) persisted after adjusting for all the above factors, metabolic covariates and use of other drugs at enrolment and during follow-up using a propensity score as well as taking non-linear associations into account (Tables 2 and 3). Combined use of statins and RAS inhibitors was consistently associated with lower risks of cancer even after removal of 682 patients followed for less than 2.5 years (Hazard ratio: 0.41, 95% CI: 0.18 to 0.94 ) and re-inclusion of 1943 patients who used RAS inhibitors or statins at baseline (0.35, 95% CI:0.22 to 0.54). In addition, combined use of RAS inhibitors and statins was also consistently associated with lower risks of a variety of site-specific cancers (supplemental table is available in the online appendix at http://diabetes.diabetesjournal.org).

Figure 1 shows the cumulative incidence of cancer in patients who used statins or RAS inhibitors alone, or both, was lower than those patients who were not exposed to statins and RAS inhibitors over the follow-up period.

**Prevention of renal cancer by RAS inhibitors in uninephrectomized rats:** We then explored the role of RAS activation in the development of cancers using the uninephrectomized (UNX) rat model. By 10 months post-operation (Figure 2), all of the 8 untreated UNX rats (100%) developed invasive renal cell carcinoma in the remnant kidney (Figure 2B). In comparison, none of

the sham rats (Figure 2A) or UNX rats treated with the ACEI-lisinopril (Figure 2C) developed renal cancer. As previously reported (27; 28), untreated UNX rats also exhibited a phenotype resembling that of type 2 diabetes, characterized by insulin resistance and pancreatic β-cell deficit, while treatment with lisinopril significantly reduced hypertrophy of the remnant kidney (28).

**Improved lipid metabolism by RAS inhibitors in uninephrectomized rats:** We used the UNX model to examine the longitudinal effect of RAS inhibition on lipid metabolism and renal function. From 3 months onwards, untreated UNX rats exhibited progressive chronic renal dysfunction, as indicated by increased total urine protein to creatinine ratio (Figure 3A). Elevated LDL-C level was observed in the untreated UNX rats from 6 months post-uninephrectomy (Figure 3B). Interestingly, treatment with ACEI largely attenuated the renal dysfunction (Figure 3A) and improved lipid metabolism (Figure 3B) in UNX animals (all $p < 0.05$ versus untreated UNX rats). Western blot assays of renal tissues revealed a 4-fold increase in the protein expression of HMGCR in the untreated UNX rats compared to the sham animals (Figure 4). Treatment with the ACEI-lisinopril normalized the expression of HMGCR (Figure 4).

**Normalization of IGF-1 signalling pathway by ACEI in uninephrectomized rats:** We then examined the protein expression of key molecules in the IGF-1 signaling pathway, which may be linked to carcinogenesis. Compared to sham animals, the protein expression of the growth inhibitory factor, IGFBP3, in the remnant kidney was reduced while the protein expression levels of growth-promoting factors, such as Akt/PKB and PKC ζ, were increased in the untreated UNX rats (Figure 5). RAS inhibition by ACEI nearly normalized the expression of these IGF-1 signal molecules (Figure 5).

## DISCUSSIONS

This study provides evidence that the combined use of RAS inhibitors and statins may be associated with greater anti-cancer effects than either class of drugs used in isolation. To explain this phenomenon, our animal data indicated that RAS blockade prevented the development of renal cell carcinoma in UNX rats via normalizing the expression of HMGCR and IGF-1 signaling pathway.

The risk associations of statin use and cancer remain controversial. In epidemiological studies, the use of statin was associated with a large relative risk reduction for cancer (37). Conversely, nearly all meta-analyses of clinical trials (13; 38) found that statins have a neutral effect on incidence of cancer. However, results from meta-analyses are often inconclusive due to heterogeneity of study design, clinical profile of patient cohorts, different definitions for outcome measures and quality of data. This is illustrated by the marked variations in cancer incidence ranging from 0.2% to 6.3% in these reported trials (39). Furthermore, the majority of clinical trials included in these meta-analyses were not conducted in diabetic populations.

The risk association of RAS inhibitors and cancer is also controversial. A meta-analysis of randomized trials of antihypertensive drugs failed to demonstrate a reduced odds of cancer with use of antihypertensive drugs, including RAS inhibitors (40). On the other hand, the Rotterdam Study reported that use of RAS inhibitors was associated with reduced cancer risk in ACE-DD genotype carriers, who are also known to have high levels of ACE (41). Two other studies (11; 16) also found that users of RAS inhibitors had lower risks of

cancer than non users. Our study further found that combined use of stains and RAS inhibitors was associated with a larger reduction in cancer risk compared with the added risk reduction associated with the use of either of the two types of drugs in isolation. In support of these clinical observations, experimental studies also indicated that RAS activation can influence carcinogenesis and tumor growth by inducing oxidative stress (42) and modulating angiogenesis, cell proliferation, immune responses and extracellular matrix formation (12). In our experimental studies, the UNX rats developed glucose intolerance and abnormal lipid metabolism and eventually renal cancer, which were all prevented by treatment with ACE inhibitors. This anti-cancer effect appears to be at least partially mediated through modulation of the HMGCR and IGF-1 signaling pathways, with the latter having complex effects on intermediary metabolism and cellular growth. The tissue activity of IGF-1 is regulated by the levels of its binding proteins as well as the number and responsiveness of its receptors. In this regard, low levels of IGFBP3 are associated with increased risk of cancer in clinical studies. On the other hand, downstream signals of the IGF-1 pathway such as Akt/PKB and PKC ζ can stimulate cell proliferation and promote cell mitosis (43). Thus, given the cancer-enhancing effects of the RAS components (12), interactions between IGF-1 signaling molecules and the tissue RAS components have been shown to stimulate the bcl-2 proto-oncogene-associated cell proliferation and to inhibit the p53 anti-oncogene-mediated cell death (44).

Cholesterol is essential for cell division and growth. In the context, IGF-1 has been shown to regulate the induction and expression of a family of genes involved in cholesterol biosynthesis (45). Thus, it is plausible that over-activation of components of the RAS, IGF-1 and HMGCR pathways may result in dysregulated growth and eventually carcinogenesis, as evidenced by 1) reduced expression of IGFBP3, 2) activation of the IGF-1 signaling pathway (Akt/PKB and PKC ζ) and 3) increased HMGCR expression in our UNX rat model. Of note, the interaction of combined use of statins and RAS inhibitors was observed for multiple cancers in humans, including cancer of "genitourinary organs", while the mechanistic exploration was made for kidney cancer in animals. In this regard, a strong association between diabetes and kidney cancer has been reported in a large cohort (46). If multiple cancers in T2DM share some common "pathogenesis", the IGF-1 signaling pathway is likely to play a role in development of multiple cancers in T2DM.

Our study has certain limitations. Firstly, the study is not a clinical trial and the findings are only hypothesis-generating. Secondly, we did not perform regular screening for cancer in this cohort due to finite resources. The use of principal discharge diagnosis to identify cancer cases may lead to the omission of a small number of cancer events. Thirdly, the current method of testing additive interaction does not allow us to quantify the interaction using dosages of statins and RAS inhibitors. Fourthly, the cohort was mainly clinic-based, albeit the overall clinical profile of patients was comparable to many community-based cohorts (47). Last but not least, the dysmetabolism observed in the animal model may not be applicable to humans, although the phenotypes exhibited by the UNX model were highly commensurate with that of T2DM.

In conclusion, we observed a synergistic effect of combined use of RAS inhibitors and statins on reducing cancer risk in T2DM, suggesting that cross-talk of RAS and lipid metabolism may play an important

role in the elevated risk of cancer in T2DM. Diabetes predisposes patients to increased risks of abnormal lipid metabolism, and elevated RAS activity is more frequent in Type 2 diabetic population than in the general population. Presumably, T2DM people with high LDL-C or hypertension would be at risk of cancer via similar mechanisms. Thus, our findings are especially important and relevant to T2DM. On the other hand, given that cross-talk between the RAS and lipid metabolism exists in the general population for the development of atherosclerosis, whether the findings of the present study would apply to the general population warrants further investigations.

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**Conflict of Interests:** None.

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**Table 1**. Clinical and biochemical characteristics of the study cohort stratified according to the occurrence of cancer during follow-up period.

| | Non-cancer (n=3970) | Cancer (n=190) | |
|---|---|---|---|
| | Median (IQR) or %(n) | Median (IQR) or %(n) | P value |
| **Baseline variables** | | | |
| Age, yr | 54(21) | 66(15) | <.0001† |
| Male gender | 45.9%(1823) | 51.6%(98) | .1263‡ |
| Smoking status | | | <.0001‡ |
| Ex-smoker | 13.6%(541) | 20.5%(39) | |
| Current smoker | 16.4%(650) | 25.8(49) | |
| Alcohol drinking status | | | <.0001‡ |
| Ex-drinker | 11.2%(443) | 21.1%(40) | |
| Current drinker | 7.7%(305) | 9.0%(17) | |
| BMI, $kg/m^2$ | 24.5(4.8) | 24.4(4.8) | .8547† |
| Duration of diabetes, yr | 5(9) | 6(9) | .0793† |
| Systolic BP, mmHg | 131(25) | 135(23) | .0011† |
| Diastolic BP, mmHg | 75(13) | 75(16) | .8312† |
| Glycated hemoglobin, % | 7.2(2.1) | 7.3(2.4) | .8346† |
| LDL-C, mmol/L | 3.20(1.20) | 3.10(1.40) | .3819† |
| HDL-C, mmol/L | 1.25(0.45) | 1.25(0.54) | .7684† |
| Triglyceride, mmol/L | 1.28(0.97) | 1.17(0.74) | .0383† |
| Total cholesterol, mmol/L | 5.19(1.30) | 5.10(1.41) | .2859† |
| ACR (mg/mmol) | 1.48(5.05) | 2.71(10.40) | <.0001† |
| eGFR, ml $min^{-1}$ 1.73 $m^{-2}$ | 109.2(38.8) | 100.0(38.1) | <.0001† |
| Prior myocardial infarction | 0.5%(18) | 2.6%(5) | <.0001‡ |
| Prior stroke | 2.7%(107) | 3.2%(6) | .7015‡ |
| Death (all-cause) | 5.8%(230) | 49.0%(93) | <.0001‡ |
| **Medications at enrolment** | | | |
| Fibrates | 2.6%(104) | 1.6%(3) | .4313‡ |
| Use of lipid lowering drug other than fibrates and statins | 0.1%(4) | 0.0%(0) | 1.0¶ |
| Antihypertensive drugs other than RAS inhibitors | 27.2%(1080) | 40.5%(77) | <.0001‡ |
| Oral anti-diabetic drugs | 60.0%(2382) | 62.6%(119) | .4328‡ |
| Insulin | 13.6%(541) | 17.4%(33) | .1441‡ |
| **Medications during follow-up period§** | | | |
| Statins only | 9.3%(368) | 3.2%(6) | .0004‡ |
| Duration of use of statins in those who used statins only, yr | 1.71(2.82) | 2.00(1.40) | |
| RAS inhibitors only | 26.1%(1036) | 27.4%(52) | .6966‡ |

| | | | |
|---|---|---|---|
| Duration of use of RAS inhibitors in those who used RAS inhibitors only, yr | 2.28(3.71) | 1.49(2.59) | |
| Both statins and RAS inhibitors | 15.8%(626) | 9.0%(17) | .00111‡ |
| Duration of combined use of statins and RAS inhibitors, yr | 1.77(3.08) | 1.16(3.22) | |
| Fibrates | 9.4%(372) | 5.3%(10) | .0555‡ |
| Lipid lowering drug other than fibrates and statins | 0.3%(12) | 0.5%(1) | .4559¶ |
| Oral anti-diabetic drugs | 82.7%(3284) | 75.8%(144) | .0142‡ |
| Insulin | 33.1%(1312) | 33.2%(63) | .9749‡ |

Abbreviations: IQR, Interquartile range; BMI, body mass index; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; BP, blood pressure; ACR, spot urine albumin:creatinine ratio; eGFR, estimated glomerular filtration rate; RAS, renin-angiotensin system and RAS inhibitors included angiotensin I converting enzyme inhibitors (ACEI) and angiotensin II receptor blockers (ARB);
†, Derived from Wilcoxon Two-Sample test;
‡, Derived from Chi-square test;
¶, Derived from Fisher's exact test;
§, From baseline (including use at baseline for all drugs except for statins) to cancer, death or censoring dates whichever came first.

**Table 2.** Hazard ratios of use of RAS inhibitors and statins for cancer in type 2 diabetes mellitus

| Exposures | Number at risk | Hazard ratio | 95% confidence interval | P value |
|---|---|---|---|---|
| **Main effect model one†** | | | | |
| Use of RAS inhibitors | 1770 | 0.52 | 0.37 to 0.74 | 0.0002 |
| Use of statins | 1056 | 0.40 | 0.25 to 0.65 | 0.0002 |
| **Main effect model two‡** | | | | |
| Use of RAS inhibitors | 1770 | 0.47 | 0.31 to 0.70 | 0.0003 |
| Use of statins | 1056 | 0.43 | 0.24 to 0.75 | 0.0035 |
| **Multiplicative interaction model one†** | | | | |
| Use of RAS inhibitors | 1770 | 0.49 | 0.34 to 0.71 | 0.0001 |
| Use of statins | 1056 | 0.26 | 0.10 to 0.65 | 0.0038 |
| Use of RAS inhibitors× use of statins | 682 | 1.98 | 0.68 to 5.75 | 0.2117 |
| **Multiplicative interaction model two‡** | | | | |
| Use of RAS inhibitors | 1770 | 0.43 | 0.27 to 0.66 | 0.0001 |
| Use of statins | 1056 | 0.26 | 0.09 to 0.74 | 0.0114 |
| Use of RAS inhibitors× use of statins | 682 | 1.99 | 0.62 to 6.43 | 0.2512 |
| **Additive interaction model one†** | | | | |
| Use of RAS inhibitors plus non-use of statins vs. others | 1088 | 0.50 | 0.35 to 0.72 | .0002 |
| Use of statins plus non-use of RAS inhibitors vs. others | 374 | 0.27 | 0.11 to 0.67 | .0049 |
| Use of RAS inhibitors plus use of statins vs. others | 643* | 0.26 | 0.15 to 0.45 | <.0001 |
| **Additive interaction model two‡** | | | | |
| Use of RAS inhibitors plus non-use of statins vs. others | 1088 | 0.43 | 0.28 to 0.67 | .0002 |
| Use of statins plus non-use of RAS inhibitors vs. others | 374 | 0.27 | 0.10 to 0.75 | .0119 |
| Use of RAS inhibitors plus use of statins vs. others | 643 | 0.22 | 0.11 to 0.43 | <.0001 |

Stratified Cox models on deciles of the likelihoods using statins and using RAS inhibitors during follow-up period were used in all the analyses. The propensity scores were calculated using logistic regression with the drug use as the dependent variable and the following variables as independent variables: age, sex, smoking status (current or ex), drinking status (current or ex), BMI, LDL-C, HDL-C, triglyceride, $HbA_{1c}$, systolic BP, $\log_{10}$ (ACR+1), eGFR, duration of diabetes, peripheral arterial disease, retinopathy, sensory neuropathy, prior myocardial infarction and prior stroke (The c-statistics was 0.79 for use of statins; and 0.80 for use of RAS inhibitors);

†, Adjusted for LDL-C related risk (i.e., <2.80 mmol/L plus albuminuria and ≥3.80 mmol/L), age, sex, BMI, smoking status (current plus ex), and alcohol drinking (current plus ex);

‡, Adjusted for LDL-C related risk (i.e., <2.80 mmol/L plus albuminuria and ≥3.80 mmol/L), age, sex, BMI, smoking status, and alcohol drinking, HDL-C, triglyceride, duration of diabetes, $HbA_{1c}$, systolic BP, eGFR and medications from enrolment to cancer, death or censoring date (oral anti-diabetic drugs, insulin and fibrates) whichever came first, and use of other antihypertensive drugs at enrolment. To avoid over-fitting, the propensity score for cancer was used for all adjustments. In addition, restricted spline functions of all continuous covariates were used to calculate the propensity score to improve adjustment for non-linear associations (The c-statistics was 0.77).

*, 39 patients who used both ACEI/ARB and statins but at different time periods were not counted as “Use of RAS inhibitors plus statins”.

**Table 3.** Additive interactions of use of RAS inhibitors and statins for the risk of cancer in type 2 diabetes mellitus

| Measures of additive interaction of RAS inhibitors with statins | Estimate | 95% confidence interval |
|---|---|---|
| Model one † | | |
| Relative excess risk due to interaction (RERI) | 0.39* | 0.09 to 0.69 |
| Attributable proportion due to interaction (AP) | 1.57* | 0.21 to 2.94 |
| Synergy index (S) | 0.66 | 0.50 to 0.86 |
| Model two ‡ | | |
| RERI | 0.53* | 0.18 to 0.86 |
| AP | 2.37* | 0.27 to 4.48 |
| S | 0.60 | 0.45 to 0.79 |

*, Statistically significant with RERI > 0, AP > 0 and S > 1 indicating additive interaction;
† and ‡, the adjusted schemes are available in Table 2.

**Figure 1.** Title: Kaplan Meier plot showing the cumulative incidences of cancer in patients with type 2 diabetes stratified by a combination of use of statins and RAS inhibitors over the follow-period (P for Log-rank test <.0001)

**Figure 2.** Title: Uninephrectomy (UNX) -induced renal cell carcinoma in remnant kidney.
Legends: Kidney tissues of 10 months post operation were obtained from sham rats (panel A), untreated UNX rats (panel B), and UNX rats treated with the ACEI-lisinopril (panel C). Periodic acid Schiff (PAS) stain demonstrates invasive renal cell carcinoma in remnant kidney of untreated UNX rats (panel B), but not of sham rats or UNX rats treated with the ACEI. Original magnification ×100.

**Figure 3.** Title: Renal dysfunction and elevated LDL cholesterol following uninephrectomy.
Legends: Compared with sham rats (square, dashed), uninephrectomized rats (UNX, circle, dark solid) progressively developed renal dysfunction, as assessed by urine protein to creatinine ratio (panel A), and hyperlipidemia (panel B), as reflected by elevated LDL cholesterol level. The proteinuria and hyperlipidemia were largely attenuated by treatment with ACEI-lisinopril (triangle, dark solid). Data are mean±SD, * $p<0.05$ versus sham and ACEI.

**Figure 4.** Title: Changes in protein expression of HMG-CoA reductase (HMGCR) in renal cortex and the effects of treatment with ACEI (lisinopril).
Legends: Renal tissue specimens were obtained after 10 months post-operation. Western blot assays revealed a 4-fold increase of HMGCR protein expression in the remnant kidney cortex of untreated UNX rats. This over-expression of HMGCR was largely normalized by treatment with ACEI-lisinopril; * $p<0.05$ versus sham and ACEI.

**Figure 5.** Title: Changes in protein expression of insulin growth factor-1 (IGF-1) signalling pathway in renal cortex and the effects of treatment with ACEI (lisinopril).
Legends: Renal tissue specimens were obtained after 10 months post-operation. Compared with sham rats, protein expression of the cancer suppressing insulin growth factor binding protein 3 (IGFBP3) was substantially diminished whereas the cancer promoting signals of Akt and PKCζ were increased in the untreated UNX rats. Treatment with ACEI largely normalized the protein expression of these key molecules in the IGF-1 signaling pathway.

**Figure 1**

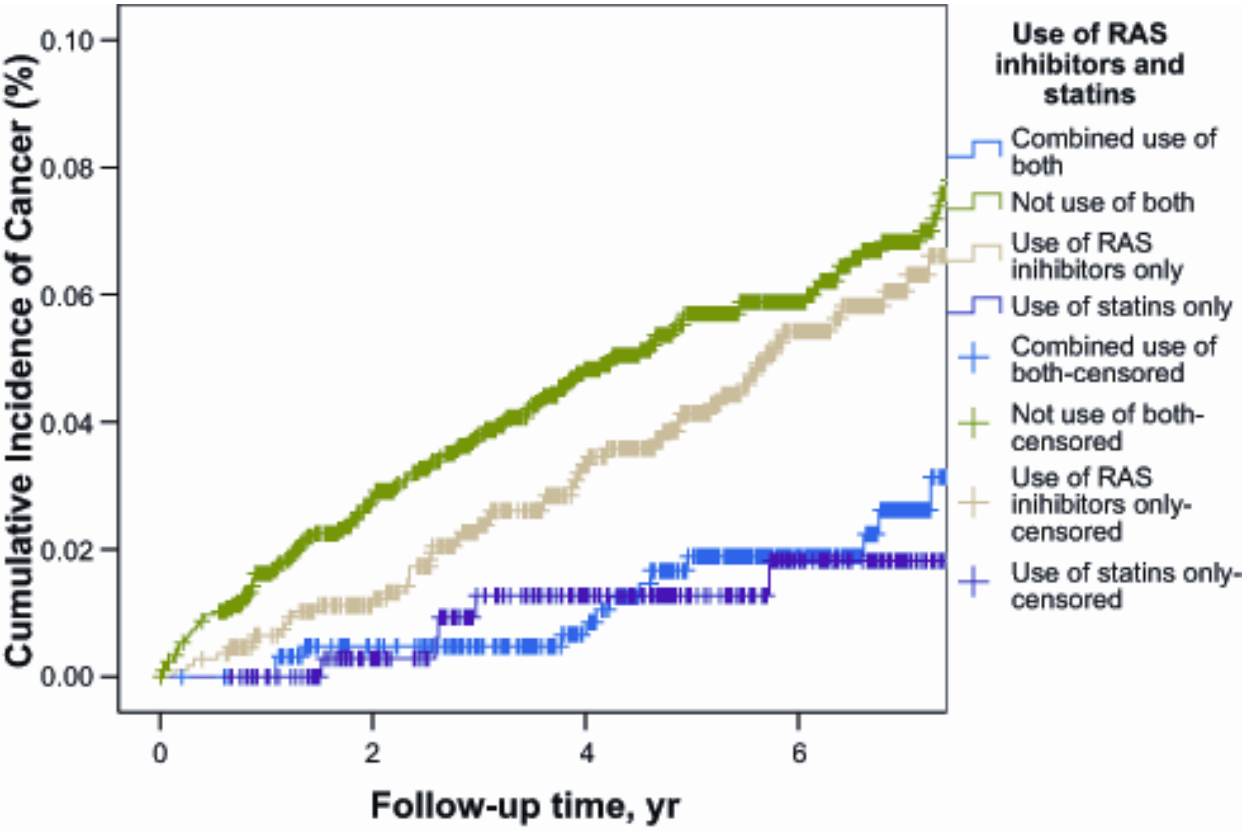


**Figure 2**

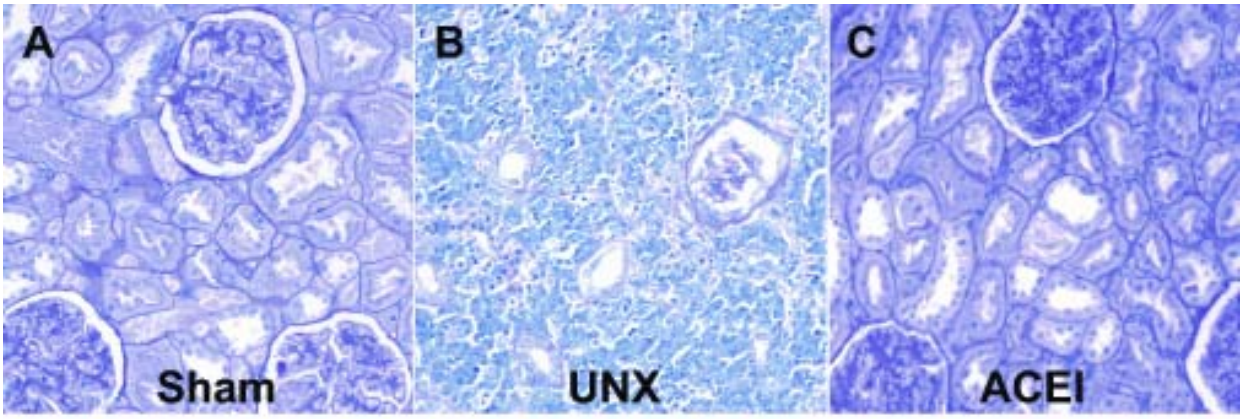

**Figure 3**

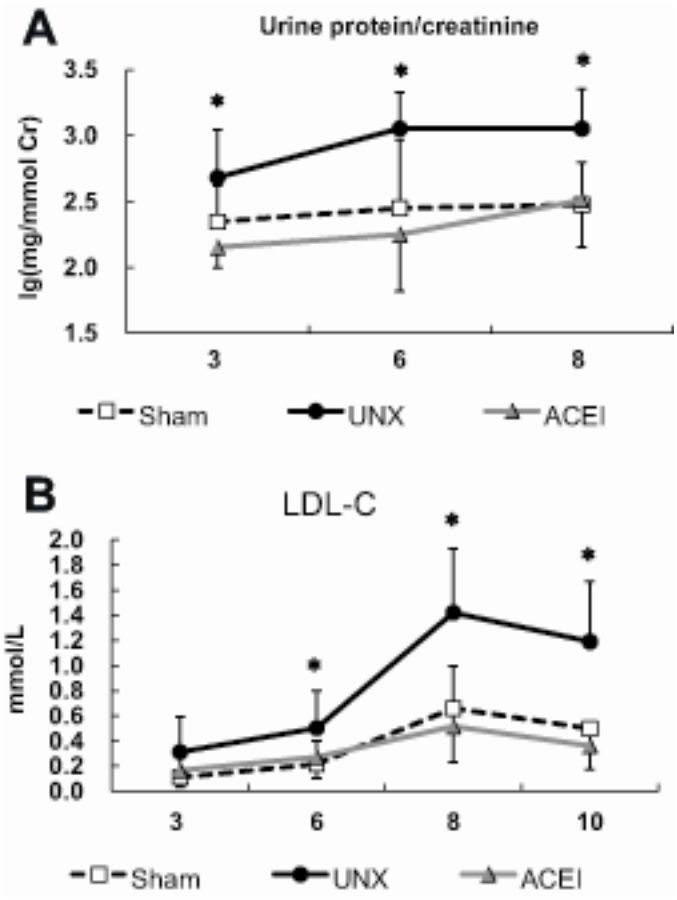
A
Urine protein/creatinine
lg(mg/mmol Cr)
3.5
3.0
2.5
2.0
1.5
3
6
8
Sham
UNX
ACEI
B
LDL-C
mmol/L
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
3
6
8
10
Sham
UNX
ACEI

**Figure 4**

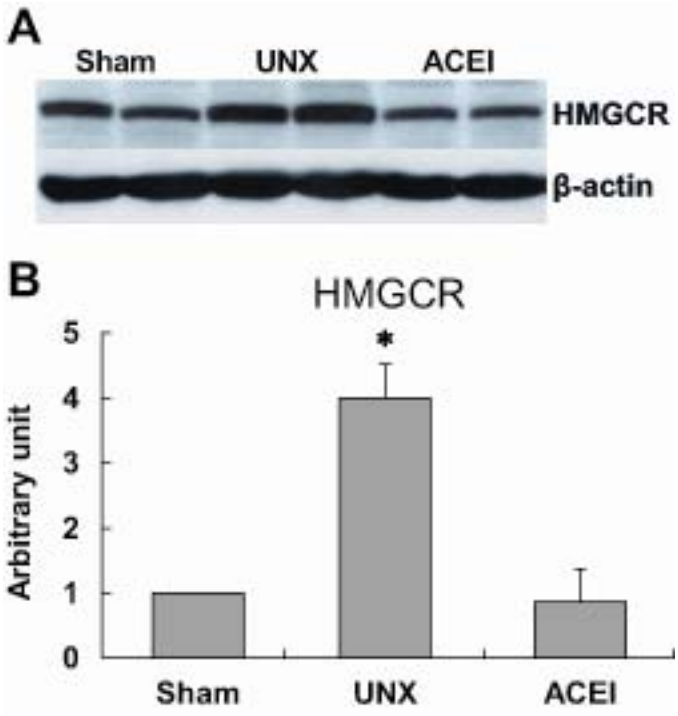


**Figure 5**