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Original article

Metformin is associated with a lower risk of non-Hodgkin lymphoma in patients with type 2 diabetes

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ABSTRACT

Background. – Whether metformin use might affect the risk of non-Hodgkin lymphoma (NHL) remained to be answered.
Methods. – A total of 610,089 newly diagnosed type 2 diabetes patients with 2 or more times of prescription of antidiabetic drugs during 1999–2009 were enrolled from Taiwan's National Health Insurance database. They were followed up for NHL incidence until December 31, 2011. Both intention-to-treat and per-protocol analyses were conducted. Cox regression incorporated with the inverse probability of treatment-weighting using propensity scores was used to estimate hazard ratios.
Results. – There were 414,783 metformin initiators and 195,306 non-metformin initiators within the initial 12-month of prescriptions of antidiabetic drugs. After a median follow-up of 5.07 years in metformin initiators and 6.78 years in non-metformin initiators, 1076 and 755 patients were diagnosed of new-onset NHL, respectively. The respective incidence was 47.74 and 57.68 per 100,000 person–years and the hazard ratio for metformin initiators versus non-metformin initiators was 0.849 (95% confidence interval 0.773–0.932) in the intention-to-treat analysis. In the per-protocol analysis, the hazard ratio was 0.706 (95% confidence interval 0.616–0.808). Sensitivity analyses after excluding patients with irregular follow-up, with an extension of minimal observation periods of 24 or 36 months, with incretin-based therapies, or in patients enrolled during 2 different periods (i.e., 1999–2003 and 2004–2009) consistently showed a lower risk among metformin initiators in both the intention-to-treat and the per-protocol analyses.
Conclusions. – Metformin use is associated with a lower risk of NHL compared with non-metformin antidiabetics.

Abbreviations

AMPK	adenosine monophosphate-activated protein kinase
ICD-9-CM	International Classification of Diseases, Ninth Revision, Clinical Modification
mTOR	mammalian target of mitomycin
NHI	National Health Insurance
NHL	non-Hodgkin lymphoma
PS	propensity score

Introduction

According to Global Cancer Statistics in 2012, there were 385,700 incident cases of non-Hodgkin lymphoma (NHL) and 199,700 patients died of NHL in that year over the world [1]. The incidence of NHL is highest in more developed countries located in Northern America, Western and Northern Europe and Australia [1]. People living in Asian countries and in Eastern Europe and Africa have the lowest rates [1]. However, the incidence of Burkitt lymphoma (a subtype of NHL) may be high among children in sub-Saharan areas [1]. Altered immune function is associated with a high risk. Therefore, NHL incidence is elevated in patients who receive immunosuppressants, suffer from autoimmune diseases or have infections of hepatitis B/C virus or human immunodeficiency virus [1–3]. Epstein-Barr virus can cause Burkitt lymphoma [1] and infection with Helicobacter pylori increases the risk of gastric

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lymphoma [4]. Studies from various groups have consistently demonstrated a strong association between diabetes and NHL [5–7]. Such an association was also supported by our previous studies that showed a higher incidence of [8] and mortality from [9] NHL among diabetes patients in Taiwan.

In Taiwan, NHL incidence is increasing [8] and NHL accounts for approximately 2.6% in males and 2.1% in females of all cancers [10]. The incidence after age-standardization increased gradually from 2.29 (men: 2.65, women: 1.88) per 100,000 population in 1980–1984 to 6.50 (men: 7.46, women: 5.52) per 100,000 population in 2000–2006 [10].

Lymphoma cells are characterized by an inactivation of adenosine monophosphate-activated protein kinase (AMPK) resulting in an enhancement of the mammalian target of mitomycin (mTOR) pathway [11]. Metformin treatment inhibits cell growth of B- and T-cell lymphoma via activation of AMPK and inhibition of the mTOR pathway [11]. However, whether the findings of these in vitro studies could be translated into a beneficial effect of metformin on NHL in humans remained to be answered. A recent population-based nested case-control study conducted in Canada using cancer registry and administrative database suggested a null association between metformin and NHL among diabetes patients [12]. The findings of this recent study remain to be confirmed because the case-control study design may suffer from methodological limitations for causal inferences.

It is recognized that studies investigating the effect of metformin on NHL are still rare and remain lacking in the Asian populations. Therefore, the present study aimed at investigating whether NHL risk could be affected by metformin use in type 2 diabetes patients by using the reimbursement database of the Taiwan's National Health Insurance (NHI).

Materials and methods

The NHI has been implemented in Taiwan since March 1995. It is a unique health care system that covers > 99% of the Taiwan's population. All in-hospitals and 93% of outpatient clinics in Taiwan have contracts with the Bureau of the NHI. The NHI reimbursement database keeps records of all disease diagnoses, prescribed medications and performed procedures. Academic research using the database can be approved after ethics review. The present study was approved number 99274.

The International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9-CM) had been used for disease coding during the study period. Diabetes in the study was defined by ICD-9-CM codes including 250.XX and NHL by codes of 200 and 202-203.

Metformin exposure was classified according to the initial 12-month period of prescriptions of antidiabetic drugs after diabetes diagnosis. Patients who had been prescribed metformin during this initial 12-month period were classified as metformin initiators [metformin (+)] and patients without any metformin prescription during the initial 12-month period were classified as non-metformin initiators [metformin (−)] [13].

A detailed description of the database was given in a previously published paper [14]. The procedures followed in creating the cohorts of metformin (+) and metformin (−) are shown in Fig. 1. A total of 778,300 patients who were newly diagnosed of diabetes from 1999 to 2009 and had been followed up and prescribed antidiabetic drugs for at least 2 times in the outpatient clinics were first selected. The following patients were then excluded:

- type 1 diabetes mellitus (n = 3667);
- missing data (n = 2566);
- diagnosis of any cancer prior to the start of follow-up (n = 76,017);
- age < 25 years at the start of follow-up (n = 7242);
- age > 75 years at the start of follow-up (n = 62,258);
- available data of exposure assessment less than 12 months (n = 16,461).

A total of 610,089 patients, among them 414,783 were metformin (+) and 195,306 were metformin (−), were used for analyses.

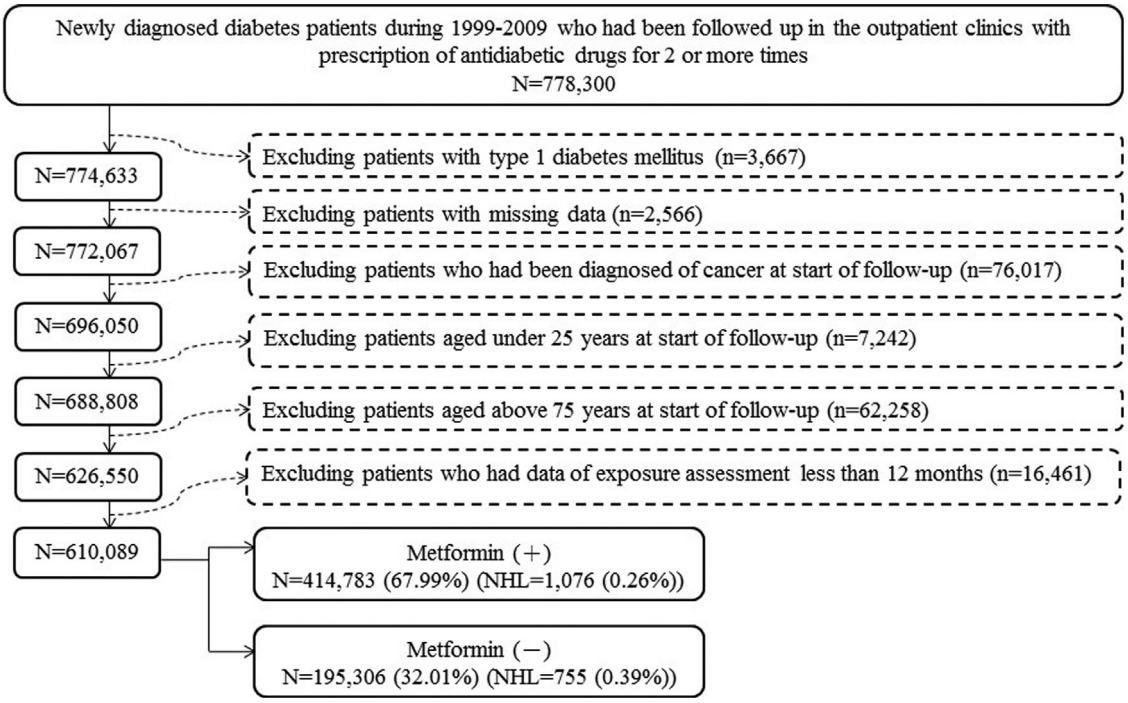

Fig. 1. Flowchart showing the procedures followed in creating a cohort of metformin initiators [Metformin (+)] and non-metformin initiators [Metformin (−)] from the reimbursement database of the National Health Insurance (NHL: non-Hodgkin lymphoma).

Potential confounders were categorized into demographic data, major comorbidities, diabetes-related complications, potential risk factors of NHL and other cancers and medications that are commonly used in diabetes patients or may affect cancer risk. Time elapsed since diabetes diagnosis was defined as the time between diabetes diagnosis and the time of the first prescription of antidiabetic drugs. The living region was classified as Taipei, Northern, Central, Southern, and Kao-Ping/Eastern. Occupation was classified as:

- class I, including civil servants, teachers, employees of governmental or private businesses, professionals and technicians;
- class II, including people without a specific employer, self-employed people and seamen;
- class III, including farmers and fishermen;
- class IV, including low-income families supported by social welfare and veterans.

Major comorbidities included hypertension, dyslipidaemia and obesity. Diabetes-related complications included nephropathy, eye disease, stroke, ischemic heart disease and peripheral arterial disease. Potential risk factors of NHL and other cancers included chronic obstructive pulmonary disease, tobacco abuse, alcohol-related diagnoses, Helicobacter pylori infection, Epstein-Barr virus infection, hepatitis B virus infection, hepatitis C virus infection, human immunodeficiency virus disease (ICD-9-CM code 042), organ transplantation (ICD-9-CM code V42), and autoimmune diseases (celiac disease: ICD-9-CM 579.0; psoriatic arthritis: ICD-9-CM 696.0; psoriasis: ICD-9-CM 696.1; systemic lupus erythematosus: ICD-9-CM 710.0; systemic sclerosis: ICD-9-CM 710.1; Sjogren's syndrome: ICD-9-CM 710.2 and rheumatoid arthritis: ICD-9-CM 714.0). The ICD-9-CM codes for other diagnoses not mentioned here can be found in a previously published paper [14]. Medications that are commonly used in diabetes patients or may affect cancer risk included angiotensin converting enzyme inhibitor/angiotensin receptor blocker, calcium channel blocker, statin, fibrate, aspirin and immunosuppressants (consistent use $\geq$ 90 days and included corticosteroids, calcineurin inhibitors and/or inosine-5'-monophosphate dehydrogenase inhibitors).

Standardized difference was calculated for each covariate as a test of balance diagnostics according to the methods proposed by Austin and Stuart, who recommended a cutoff value of $>$ 10% as an indication of potential confounding [15].

To emulate a target trial comparing metformin versus non-metformin antidiabetics on the risk of NHL, both intention-to-treat and per-protocol analyses were conducted. For intention-to-treat analyses, the number of new cases of NHL diagnosed during follow-up was the numerator of the incidence. The denominator was expressed as person-years. Follow-up started at the end of the initial 12-month period used for exposure assessment, and ended at NHL diagnosis, death, or the last reimbursement record until December 31, 2011, whichever occurred first, with no exclusion according to switching to or adding other antidiabetic drugs thereafter.

In the per-protocol analyses, patients who did not adhere to the assigned treatment within the 12-month period of exposure assessment were first excluded and others were followed up starting at the end of the 12-month period as done in the intention-to-treat analyses. Follow-up in the per-protocol analyses ended at NHL diagnosis, death or the last reimbursement record, or when non-adherence to the assigned treatment occurred [i.e., at the time of addition of metformin in the metformin (–) group, and at the time of addition of non-metformin antidiabetic drug in the metformin (+) group], whichever occurred first until December 31, 2011.

Kaplan-Meier curves for NHL-free probability were plotted for metformin (+) and metformin (–) according to intention-to-treat and per-protocol analyses, respectively. Log-rank test was used to test the significance in the two different groups of metformin exposure.

In consideration that the distribution of baseline characteristics might differ between metformin (+) and metformin (–), hazard ratios and their 95% confidence intervals for metformin (+) versus metformin (–) were estimated by Cox regression constructed with the inverse probability of treatment-weighting using propensity scores (PS), as recommended by Austin to reduce the potential confounding by differences in characteristics [16]. Logistic regression was used to create PS derived from the date of the start of follow-up plus all baseline characteristics in Table 1. The inclusion of the date of start of follow-up accounted partly for some unknown risk factors such as changes in treatment guidelines or the introduction of novel therapeutic agents during the long inclusion period.

The following sensitivity analyses were conducted to examine the consistency of the findings. First, patients were censored from the time 4 months and 6 months, respectively, have elapsed since the last prescription. Because the Bureau of the NHI allows drug prescription for no more than 3 months in each outpatient visit, censoring patients at these time points excluded the follow-up time of irregular drug refills in the calculation of person-years. Second, patients who had been followed up for $<$ 24 and $<$ 36 months, respectively, were excluded. These analyses excluded the possible interpretation of NHL cases diagnosed within 24 months and 36 months of follow-up, respectively, as an effect of treatment assignment [13]. Third, patients who happened to be treated with incretin-based therapies (many of them were introduced into Taiwan during the follow-up period) were excluded. Fourth, patients enrolled during 1999–2003 and 2004–2009 were analyzed separately. The analyses of patients enrolled during two different periods of time further examined whether the findings might be affected by some unknown risk factors such as changes in treatment guidelines or the introduction of novel therapeutic agents.

SAS statistical software (version 9.3, SAS Institute, Cary, NC) was used for statistical analyses, with $P < 0.05$ being considered as statistically significant.

Results

Table 1 compares the baseline characteristics of metformin (–) and metformin (+). The two groups varied in characteristics of age, time elapsed since diabetes diagnosis, occupation, dyslipidaemia, obesity, eye disease and statin with values of standardized difference $>$ 10%. The first antidiabetic drugs used in the metformin (–) group were mainly sulfonylureas (85.63%), followed by acarbose (5.32%), insulin (5.15%), meglitinide (3.83%), rosiglitazone (1.42%), pioglitazone (0.93%) and sitagliptin (0.14%).

The median duration of follow-up for metformin (–) and metformin (+) was 6.78 and 5.07 years, respectively, in the intention-to-treat analyses; and was 2.38 and 4.58 years, respectively, in the per-protocol analyses. Fig. 2 shows the Kaplan-Meier curves comparing NHL-free probability in metformin (+) and metformin (–) groups in the intention-to-treat (Fig. 2A) and in the per-protocol (Fig. 2B) analyses, respectively. The log-rank test suggested a lower risk of NHL associated with metformin use in both analyses.

Table 2 shows the incidence rates of NHL and the hazard ratios comparing metformin (+) versus metformin (–). Both the intention-to-treat analysis and the per-protocol analysis suggested a significantly lower risk of NHL among metformin (+) group while

Table 1
Baseline characteristics in non-metformin initiators and metformin initiators.

Variable	Metformin (–) (n = 195306)		Metformin (+) (n = 414783)		Standardized difference
	n	%	n	%	
Demographic data					
Age[a] (years)	55.61	10.92	53.85	11.07	−16.39
Time elapsed since diabetes diagnosis[a] (years)	1.61	1.38	1.83	1.54	17.77
Sex (men)	108070	55.33	228303	55.04	−0.14
Occupation[b]					
I	76442	39.14	173894	41.92	
II	42149	21.58	94215	22.71	3.22
III	44014	22.54	75968	18.32	−11.15
IV	32701	16.74	70706	17.05	0.76
Living region					
Taipei	60319	30.88	143892	34.69	
Northern	21872	11.2	53148	12.81	5.19
Central	34916	17.88	74326	17.92	0.76
Southern	34639	17.74	62944	15.18	−7.52
Kao-Ping and Eastern	43560	22.3	80473	19.4	−7.84
Major comorbidities					
Hypertension	115512	59.14	250686	60.44	3.94
Dyslipidaemia	91557	46.88	236370	56.99	21.82
Obesity	3837	1.96	18177	4.38	13.29
Diabetes-related complications					
Nephropathy	26286	13.46	51212	12.35	−2.56
Eye disease	8072	4.13	29763	7.18	13.14
Stroke	31085	15.92	63101	15.21	−1.31
Ischemic heart disease	53609	27.45	112417	27.1	0.17
Peripheral arterial disease	19779	10.13	45226	10.9	3.43
Potential risk factors of non-Hodgkin lymphoma and other cancers					
Chronic obstructive pulmonary disease	61853	31.67	138049	33.28	4.11
Tobacco abuse	2112	1.08	7487	1.81	6.19
Alcohol-related diagnoses	9028	4.62	20151	4.86	1.61
History of Helicobacter pylori infection	28737	14.71	65870	15.88	3.88
Epstein-Barr virus-related diagnoses	923	0.47	2118	0.51	0.68
Hepatitis B virus infection	1934	0.99	6698	1.61	5.67
Hepatitis C virus infection	4799	2.46	10726	2.59	1.20
Human immunodeficiency virus disease	99	0.05	211	0.05	0.13
Organ transplantation	430	0.22	520	0.13	−2.15
Autoimmune diseases	10199	5.22	24644	5.94	3.53
Medications that are commonly used in diabetes patients or may affect cancer risk					
Angiotensin converting enzyme inhibitor/angiotensin receptor blocker	88148	45.13	195569	47.15	5.02
Calcium channel blocker	87182	44.64	173559	41.84	−5.24
Statin	50026	25.61	137451	33.14	17.35
Fibrate	43828	22.44	101518	24.47	5.43
Aspirin	68807	35.23	154083	37.15	4.85
Immunosuppressants	6510	3.33	11866	2.86	−2.61

[a] Age and time elapsed since diabetes diagnosis are expressed as mean and standard deviation.
[b] Refer to "Materials and methods" for the classification of occupation.

compared to metformin (–) group. The hazard ratio was 0.849 (95% confidence interval: 0.773–0.932) in the intention-to-treat analysis and was 0.706 (95% confidence interval: 0.616–0.808) in the per-protocol analysis.

All sensitivity analyses in Table 3 supported the finding of a lower risk of NHL associated with metformin use in the main analyses shown in Table 2.

Discussion

The present study using a real-world database to emulate a clinical trial supported a significantly lower risk of NHL associated with metformin use compared with non-metformin antidiabetics in patients with type 2 diabetes mellitus (Fig. 2, Tables 2 and 3).

The mechanisms of a potential preventive effect of metformin on NHL remains to be answered but could be explained by some of its well-recognized anticancer effects. Metformin inhibits the mitochondrial respiratory-chain complex 1 and activates the liver kinase B1/AMPK pathway, resulting in the inhibition of hepatic gluconeogenesis [17]. Metformin also inhibits lipogenesis and improves insulin signalling, resulting in lower circulating insulin levels [17]. It displays many anticarcinogenic effects in in vitro and in vivo studies by activating the liver kinase B1/AMPK pathway, which in turn inhibits the mTOR pathway [18]. Metformin may eradicate cancer stem cells and activate the immune system to kill cancer cells [18]. Protein synthesis is inhibited in cancer cells treated with metformin and the cell cycle is arrested followed by apoptosis [18].

Although randomized clinical trials are considered the best study design to make causal inferences, they are not always ethical or feasible to be conducted for providing timely answers to clinical questions [19]. In recent years, analyses of big observational data, especially electronic medical records, are increasingly used for such purposes. Novel analytical methods are being developed to minimize potential limitations and to expand the usefulness of big observational data. One recent approach recommended is to analyze observational data as clinical trials by estimating the intention-to-treat hazard ratios and the per-protocol hazard ratios [19,20].

The "per-protocol" analysis stops following the patients when they deviate from their initial treatment assignment and analyzes only patients who adhere to the assigned treatment [19]. As

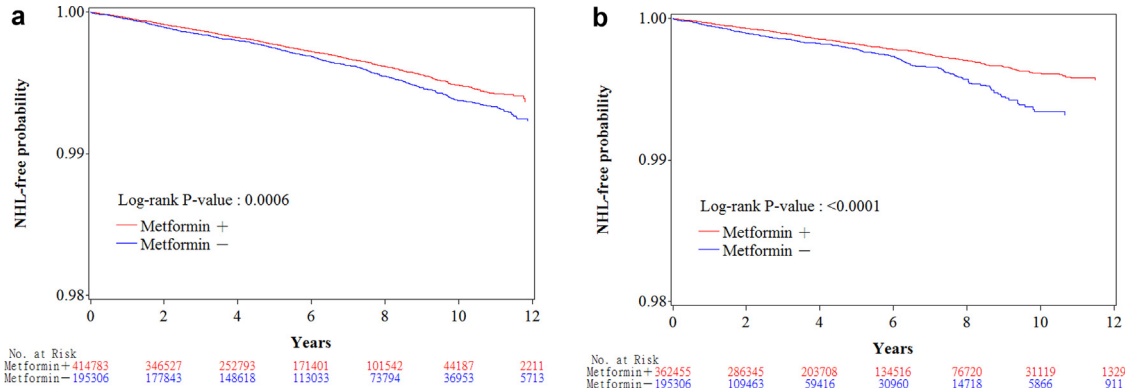

Fig. 2. Kaplan-Meier curves comparing non-Hodgkin lymphoma (NHL)-free probability in metformin initiators (Metformin+) and non-metformin initiators (Metformin–) in the intention-to-treat (A) and per-protocol (B) analyses.

Table 2
Incidence rates of non-Hodgkin lymphoma and hazard ratios comparing metformin initiators versus non-metformin initiators.

Model	Incident case number of NHL	Cases followed	Person-year	Incidence rate (per 100,000 person-years)	Hazard ratio[a] (95% confidence interval)	*P* value
Intention-to-treat						
Metformin (–)	755	195306	1308916.99	57.68	1.000	
Metformin (+)	1076	414783	2253700.87	47.74	0.849 (0.773–0.932)	0.0006
Per-protocol						
Metformin (–)	312	195306	620154.54	50.31	1.000	
Metformin (+)	665	362455	1822528.91	36.49	0.706 (0.616–0.808)	<0.0001

NHL: non-Hodgkin lymphoma.
[a] Cox regression was constructed with the inverse probability of treatment-weighting, using propensity scores derived from variables in Table 1 plus the date of start of follow-up.

pointed out by Danaei et al., "imperfect adherence may move the intention-to-treat effect towards the null" in studies comparing therapy to no therapy [19]. The larger magnitude of a lower risk of NHL in the metformin (+) group in the per-protocol analyses than in the intention-to-treat analyses in the present study (Tables 2 and 3) supported the statement of Danaei et al. [19]. Therefore, patients who adhered to metformin therapy might have a greater benefit of protection on NHL. Although the potential benefit of metformin on NHL in the diabetes patients requires additional confirmation, such a benefit of metformin is worthy of exploration in both the diabetes patients and the non-diabetes individuals because metformin is safe and cheap without a potential risk of hypoglycaemia when used alone.

Some substantial differences may exist between a hypothetical randomized trial and a non-randomized observational study using electronic medical records to emulate the target trial [19]. First, treatment was not randomly assigned in observational studies and confounding by indication as a result of remarkable differences in baseline characteristics between treatment and non-treatment group is possible. To minimize such a possible effect, Cox regression was constructed with the inverse probability of treatment-weighting, using PS derived from variables in Table 1 plus the date of start of follow-up. Second, clinical trials usually exclude individuals with the target outcome at baseline by a sequence of systematic physical or laboratory examinations, but these would not have been done in an observational study and many of the cases might have been undiagnosed at baseline [19]. To reduce such a possible effect, this study excluded patients with a diagnosis of NHL before the start of follow-up (Fig. 1, Table 2), followed by sensitivity analyses with additional exclusion of patients followed for < 24 months and < 36 months, respectively (Table 3). Third, an observational study can never be blinded as a hypothetical double-blind randomized trial can be. The awareness of treatment assignment in both patients and doctors might have affected their behaviour. Therefore, the outcome estimate might have resulted from both the pharmacological effects and behavioural changes. However, unlike outcome of cardiovascular disease that may be significantly affected by behavioural changes including diet, smoking and exercise etc., the investigated outcome of NHL in the present study would be less impacted by such an "open-labelled" study design. Fourth, in the real-world, the probability of non-adherence to the assigned treatment is expected to be greater than in a clinical trial. This could be more likely in the metformin (–) group in the present study because metformin has been recommended as a first-line therapy in recent years due to its potential beneficial effects on cardiovascular disease and cancer. Therefore, metformin might have replaced other non-metformin drugs or been added to the metformin (–) group as a combination therapy at a later time even though it had not been prescribed during the first 12-month period of the initiation of antidiabetic treatment in the study. To examine whether this really happened in the database, we calculated the non-adherence rates in the metformin (–) and metformin (+) groups with regards to the duration after follow-up for 2, 4, 6, 8, 10 and 12 years. The respective non-adherence rates in the metformin (–) group were 38.4%, 60.0%, 72.6%, 80.1%, 84.1% and 84.1%; and were 17.4%, 19.4%, 21.5%, 24.4%, 29.6% and 39.9% in the metformin (+) group. This could also explain the more remarkable reduction of follow-up period from intention-to-treat analyses to per-protocol analyses in the metformin (–) group (a reduction from 6.78 years to 2.38 years) than the metformin (+) group (from 5.07 years to 4.58 years).

Table 3
Sensitivity analyses.

Model	Incident case number of NHL	Cases followed	Person-year	Incidence rate (per 100,000 person–years)	Hazard ratio[a] (95% confidence interval)	*P* value
I. Censoring patients from the time 4 months have elapsed since the last prescription						
Intention-to-treat						
Metformin (–)	688	195306	1171969.78	58.70	1.000	
Metformin (+)	904	414783	1949913.90	46.36	0.812 (0.735–0.897)	< 0.0001
Per-protocol						
Metformin (–)	306	195306	571914.29	53.50	1.000	
Metformin (+)	559	362455	1568184.94	35.65	0.648 (0.563–0.746)	< 0.0001
II. Censoring patients from the time 6 months have elapsed since the last prescription						
Intention-to-treat						
Metformin (–)	691	195306	1191654.57	57.99	1.000	
Metformin (+)	943	414783	1998871.58	47.18	0.837 (0.759–0.924)	0.0004
Per-protocol						
Metformin (–)	302	195306	577895.19	52.26	1.000	
Metformin (+)	584	362455	1612424.33	36.22	0.672 (0.584–0.773)	< 0.0001
III. Excluding patients followed up for < 24 months						
Intention-to-treat						
Metformin (–)	556	177813	1287757.80	43.18	1.000	
Metformin (+)	740	346391	2167498.70	34.14	0.861 (0.771–0.962)	0.0081
Per-protocol						
Metformin (–)	160	177813	602447.20	26.56	1.000	
Metformin (+)	442	310891	1763562.91	25.06	0.743 (0.619–0.891)	0.0014
IV. Excluding patients followed up for < 36 months						
Intention-to-treat						
Metformin (–)	469	164407	1254045.45	37.40	1.000	
Metformin (+)	600	298855	2049233.78	29.28	0.862 (0.764–0.974)	0.0168
Per-protocol						
Metformin (–)	122	164407	578169.84	21.10	1.000	
Metformin (+)	351	270950	1676257.12	20.94	0.712 (0.579–0.875)	0.0013
V. Excluding patients who had been treated with incretin-based therapies during follow-up						
Intention-to-treat						
Metformin (–)	722	167018	1086386.99	66.46	1.000	
Metformin (+)	1029	343044	1794063.83	57.36	0.882 (0.802–0.971)	0.0103
Per-protocol						
Metformin (–)	310	167018	549117.05	56.45	1.000	
Metformin (+)	641	292699	1397915.08	45.85	0.796 (0.694–0.912)	0.0010
VI. Patients enrolled during 1999–2003						
Intention-to-treat						
Metformin (–)	553	110596	919743.18	60.13	1.000	
Metformin (+)	710	175567	1383787.45	51.31	0.863 (0.772–0.964)	0.0094
Per-protocol						
Metformin (–)	213	110596	392513.60	54.27	1.000	
Metformin (+)	431	158915	1143481.84	37.69	0.669 (0.565–0.791)	< 0.0001
VII. Patients enrolled during 2004–2009						
Intention-to-treat						
Metformin (–)	202	84710	389173.82	51.90	1.000	
Metformin (+)	366	239216	869913.42	42.07	0.828 (0.695–0.985)	0.0336
Per-protocol						
Metformin (–)	99	84710	227640.94	43.49	1.000	
Metformin (+)	234	203540	679047.07	34.46	0.794 (0.626–1.005)	0.0553

NHL: non-Hodgkin lymphoma.
[a] Cox regression was constructed with the inverse probability of treatment-weighting, using propensity scores derived from variables in Table 1 plus the date of start of follow-up.

Other common methodological limitations in the analyses of observational data included prevalent user bias and immortal time bias. These have also been carefully addressed in the study. By including only patients with new-onset type 2 diabetes mellitus and new users of metformin, prevalent user bias can be prevented. Immortal time is the follow-up time when the outcome cannot occur. Immortal time bias can be introduced when either the treatment status or the follow-up time is inappropriately assigned [21]. Misdiagnosis of diabetes and misclassification of treatment status would be less likely when the study enrolled only patients who had received 2 or more prescriptions of antidiabetic drugs (Fig. 1). Furthermore, the findings were consistent when a minimal follow-up time has been extended to 24 months or 36 months in sensitivity analyses (Table 3). The immortal time between diabetes diagnosis and the start of treatment with antidiabetic drugs was actually not included in the calculation of the follow-up person-years and this "time elapsed since diabetes diagnosis" has also been considered in the creation of the PS (Table 1). Lévesque et al. described another type of immortal time that may happen during the waiting period for prescriptions to be refilled when a patient is discharged from the hospital [21]. It is worthy to stress that this would not happen in Taiwan because all discharge medications can be directly obtained from the hospital at the time of discharge.

This study has merits of using a nationwide database that covers > 99% of the population. Therefore, the findings can be readily generalized to the whole population. The possibility of self-reporting bias was reduced by using existing medical records. For the following reasons, detection bias because of different socioeconomic classes was less likely in Taiwan when compared to studies conducted in many other countries. First, the Bureau of the NHI considers cancer as a catastrophic illness and cancer patients can be waived of most medical co-payments. Second, the drug cost-sharing is low or can be waived in patients with low-

income, in veterans or when the patients receive drug refills for chronic disease.

There are some potential limitations in the study. First, biochemical data and measurement data of some confounders like lifestyle, dietary intake, nutritional status, anthropometric factors, smoking, alcohol, family history and genetic parameters are lacking. Second, the study could not differentiate the different subtypes of NHL. Third, the information on the pathology, grading and staging of NHL was not available.

Conclusions

A significantly lower risk of NHL is associated with metformin use compared with non-metformin antidiabetics in both the intention-to-treat analyses and the per-protocol analyses in this real-world observational study. Future studies to confirm the findings in other ethnicities and in non-diabetes individuals are required.

Declaration statements

This study was approved by the Institutional Review Board of the National Health Research Institutes (approval number 99274). Individuals in the NHI database were de-identified and no informed consent was required according to local regulations. For the protection of privacy, public availability of the data is not permitted.

Author contributions

C-H.T. researched data and wrote manuscript.

Disclosure of interest

The author declares that he has no competing interest.

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References

[1] Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. CA Cancer J Clin 2015;65:87–108.
[2] Dalia S, Dunker K, Sokol L, Mhaskar R. Hepatitis B seropositivity and risk of developing multiple myeloma or Hodgkin lymphoma: a meta-analysis of observational studies. Leuk Res 2015;39:1325–33.
[3] De Re V, De Zorzi M, Caggiari L, Lauletta G, Tornesello ML, Fognani E, et al. HCV-related liver and lymphoproliferative diseases: association with polymorphisms of IL28B and TLR2. Oncotarget 2016;7:37487–9.
[4] Laur AM, Floch P, Chambonnier L, Benejat L, Korolik V, Giese A, et al. Regulatory T cells may participate in Helicobacter pylori persistence in gastric MALT lymphoma: lessons from an animal model. Oncotarget 2016;7:3394–402.
[5] Dankner R, Boffetta P, Balicer RD, Boker LK, Sadeh M, Berlin A, et al. Time-dependent risk of cancer after a diabetes diagnosis in a cohort of 2.3 million adults. Am J Epidemiol 2016;183:1098–106.
[6] Engels EA, Parsons R, Besson C, Morton LM, Enewold L, Ricker W, et al. Comprehensive evaluation of medical conditions associated with risk of non-Hodgkin lymphoma using Medicare Claims (“MedWAS”). Cancer Epidemiol Biomarkers Prev 2016;25:1105–13.
[7] Castillo JJ, Mull N, Reagan JL, Nemr S, Mitri J. Increased incidence of non-Hodgkin lymphoma, leukemia, and myeloma in patients with diabetes mellitus type 2: a meta-analysis of observational studies. Blood 2012;119:4845–50.
[8] Tseng CH. Diabetes and non-Hodgkin’s lymphoma: analyses of prevalence and annual incidence in 2005 using the National Health Insurance database in Taiwan. Ann Oncol 2012;23:153–8.
[9] Tseng CH. Diabetes, insulin use, and non-Hodgkin lymphoma mortality in Taiwan. Metabolism 2012;61:1003–9.
[10] Chiang CJ, Chen YC, Chen CJ, You SL, Lai MS. Taiwan Cancer Registry Task Force. Cancer trends in Taiwan. Jpn J Clin Oncol 2010;40:897–904.
[11] Shi WY, Xiao D, Wang L, Dong LH, Yan ZX, Shen ZX, et al. Therapeutic metformin/AMPK activation blocked lymphoma cell growth via inhibition of mTOR pathway and induction of autophagy. Cell Death Dis 2012;3:e275.
[12] Ye X, Zhang G, Righolt C, Johnston JB, Banerji V, Gibson SB, et al. Metformin is not associated with incidence risk of non-Hodgkin lymphomas among diabetic patients. Cancer Epidemiol Biomarkers Prev 2018;27:610–2.
[13] Tsilidis KK, Capothanassi D, Allen NE, Rizos EC, Lopez DS, van Veldhoven K, et al. Metformin does not affect cancer risk: a cohort study in the U.K. clinical practice research datalink analyzed like an intention-to-treat trial. Diabetes Care 2014;37:2522–32.
[14] Tseng CH. Metformin is associated with a lower risk of colorectal cancer in Taiwanese patients with type 2 diabetes: a retrospective cohort analysis. Diabetes Metab 2017;43:438–45.
[15] Austin PC, Stuart EA. Moving towards best practice when using inverse probability of treatment weighting (IPTW) using the propensity score to estimate causal treatment effects in observational studies. Stat Med 2015;34:3661–79.
[16] Austin PC. The performance of different propensity score methods for estimating marginal hazard ratios. Stat Med 2013;32:2837–49.
[17] Viollet B, Guigas B, Sanz Garcia N, Leclerc J, Foretz M, Andreelli F. Cellular and molecular mechanisms of metformin: an overview. Clin Sci (Lond) 2012;122:253–70.
[18] Kourelis TV, Siegel RD. Metformin and cancer: new applications for an old drug. Med Oncol 2012;29:1314–27.
[19] Danaei G, Rodríguez LA, Cantero OF, Logan R, Hernán MA. Observational data for comparative effectiveness research: an emulation of randomised trials of statins and primary prevention of coronary heart disease. Stat Methods Med Res 2013;22:70–96.
[20] Danaei G, García Rodríguez LA, Cantero OF, Logan RW, Hernán MA. Electronic medical records can be used to emulate target trials of sustained treatment strategies. J Clin Epidemiol 2018;96:12–22.
[21] Lévesque LE, Hanley JA, Kezouh A, Suissa S. Problem of immortal time bias in cohort studies: example using statins for preventing progression of diabetes. BMJ 2010;340:b5087.