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Statin use and risk of liver cancer: an update meta-analysis

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Key words: *Statin; Liver cancer; Cancer Prevention; Meta-analysis.*

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Abstract

Objective: Statins are commonly prescribed cholesterol-lowering drugs. Preclinical studies suggest that statins may possess cancer preventive properties. The primary objective of this meta-analysis was to determine the association between statin use and risk of liver cancer.

Design: Meta-analysis.

Setting: International.

Participants: A comprehensive literature search of PubMed, BIOSIS Previews, Web of Science, EMBASE, and EBSCO was conducted through March 2014. The effect estimate was reported as pooled relative risk (RR) with 95% confidence intervals (CIs), using the random-effects model. The test of heterogeneity, publication bias and subgroup analyses were also performed.

Results: A total of 14 (3 RCT, 5 cohort, and 6 case-control) studies were qualified for the meta-analysis, involving 1,779,630 participants with 35,775 liver cancer cases. Our results indicated a significant risk reduction of liver cancer among all statin users ((RR 0.58, 95% CIs 0.51–0.67). The difference of study design, baseline risk and confounding adjustment can partly explained the significant heterogeneity found in the overall analysis (I^2 = 59%, P=0.002). No evidence of publication bias was observed. Similar results were also found in the subgroup of lipophilic statin use (RR 0.57, 0.50–0.65; I^2=40%, P=0.13) and higher cumulative dosage of statin use (RR 0.54, 0.38-0.77; I^2=85%, P<0.00001).

Conclusions: This meta-analysis suggests that there is a significant inverse

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association between statin use and risk of liver cancer, however, some confounders might overestimate this preventive effect of statins.

Key words: *Statin; Liver cancer; Cancer Prevention; Meta-analysis.*

Strengths and limitations of this study

Statins are commonly prescribed cholesterol-lowering drugs. In this comprehensive meta-analysis, we demonstrate that the statin use is associated with a significant reduction of liver cancer risk.

The difference of study design, baseline risk and confounding adjustment can partly explained the significant heterogeneity found in the overall analysis.

Some confounders, such as adjust factor of original studies, and indication of statin use, might overestimate the preventive effect of statins on liver cancer.

Further studies are needed to investigate the efficacy of statins in the prevention and treatment of liver cancer.

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INTRODUCTION

Statins are inhibitors of 3-hydroxy-3-methylglutaryl coenzyme-A (HMG-CoA) reductase and widely used to reduce the plasma cholesterol level and the risk of cardiovascular events.[1] Although there is a concern over their possible carcinogenicity raised in rodent studies,[2] preclinical studies indicate that statins have anticancer properties *in vitro* and *in vivo*, through inhibiting angiogenesis, inducing apoptosis, and suppressing tumor growth and metastasis.[3-5] However, high concentrations are typically required to induce these effects, raising questions concerning the therapeutic relevance of statins with cancer.[6] Meanwhile, there are inconsistent results from clinical studies aiming at determining whether statins indeed reduce the risk of cancer at regular daily doses for cardiovascular event prevention. Moreover, several meta-analyses have indicated that there was no association between statin use and the risk of overall cancer,[7-10] or cancer of breast,[11] stomach,[12] or pancreas.[13] There is only a modest association between the statin use and the risk of prostate cancer[14] and colorectal cancer.[15]

In contrary to previously reported studies, several recent studies reported encouraging benefits for risk reduction of liver cancer among all statin users. Of note, in a previously reported meta-analysis of ten studies, Singh *et al*. found a significant inverse association between the statin use and the risk of hepatocellular carcinoma (HCC).[16] Considering the recently published evidences, the present meta-analysis was designed to further evaluate the association between the statin use and the risk of liver cancer, by a comprehensive literature search and more subgroup analyses based on a

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large population base. Our results demonstrated the benefits of reducing liver cancer risk in statin users with regular daily doses for prevention of cardiovascular events, which may have a significant translational potential in the clinic. However, some confounders might overestimate this preventive effect of statins.

MATERIALS AND METHODS

Literature Search strategy

This meta-analysis was conducted following the PRISMA guidelines.[17]

The systematic computerized search for eligible studies was performed on the database of PubMed, BIOSIS Previews, Web of Science, EMBASE, and EBSCO, covering all studies published from their inception to March 5, 2014. The following terms were searched with both the subjects (MeSH terms) and text-word search strategies: "(Statin OR HMG-CoA reductase inhibitors OR Atorvastatin OR Cerivastatin OR Fluvastatin OR Lovastatin OR Pravastatin OR Rosuvastatin OR Simvastatin) AND (Hepatocellular OR Hepatic OR Intrahepatic OR Interlobular OR Liver) AND (Carcinoma OR Sarcomas OR Angiosarcoma OR Cancer OR Neoplasm). Additionally, the relevant reviews and retrieved articles were searched manually for more eligible studies.

Study selection

The inclusion criteria were: (1) randomized controlled trial (RCTs), cohort studies or case-control studies; (2) original studies that assessed the effect of statin use on the risk of liver cancer, compared with placebo or no treatment; and (3) liver cancer cases were identified according to the International Classification of Diseases codes (ICD).

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The exclusion criteria were: (1) study design not meeting the inclusion criteria; (2) studies without estimate of relative risk (risk ratio, RR) of liver cancer, or liver cancer incidence by statin use status; or (3) studies with duplicated reports.

Data extraction

Two independent investigators (M. Shi and X.B. Cui) extracted data from the eligible studies using a predefined data collection form. The differences of data extraction were resolved by consensus referring back to the original article. The extracted information included: (1) Studies: first author, year of publication, study design, location, patient populations, period, and follow-up; (2) Statins: type, dosage or duration of statin use; (3) liver cancer: case identification, incidence by statin use status, crude RR with 95% confidence intervals (CIs), adjusted RR reflecting the greatest degree of control for potential confounders, and confounders for adjustment (including variables for matching). When the RR were not available, the RR with 95% CIs were calculated from the raw data provided.

We extracted different measurements of effect estimates from original studies, such as Relative Risk, Odds Ratio, Hazard Ratio, and Observed/Expected ratio. In this analysis, these different measurements were found to provide similar estimates of RR, presumably due to the fact that the incidence of liver cancer was very low in most studies.

Methodological quality assessment

Of note, the included RCTs were pooled analyses or secondary analysis of other RCTs, therefore, it is inappropriate to assess the methodological quality. The methodological

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quality of cohort and case-control studies were assessed on the Newcastle-Ottawa Scale,[18] including eight items that were categorized three categories: selection (three items, one star each), comparability (one item, up to two stars), and exposure/outcome (three items, one star each). A "star" presents a "high" quality choice of each items.

Statistical analysis

The overall meta-analysis was performed first, followed by the subgroup analyses, based on study design, study location, confounding adjustment, and baseline risk of liver cancer. Meanwhile, we conducted subgroup analyses based on studies which reported different RR estimate for use of lipophilic statins and higher cumulative dosage of statin, when appropriate data were available.

To take into account the heterogeneity and provide a more conservative estimate, the inverse variance method was used to estimate the pooled RR and corresponding 95% CIs, and data were pooled using a random effects model. Heterogeneity was assessed using the Chi-squared statistic (P) together with the Higgins I-squared statistic (I^2).[19] Test for subgroup differences was carried out to characterize possible sources of statistical heterogeneity. Publication bias was assessed using the Begg's test and the Egger's test.[20] A P-values of 0.10 was used to determine statistically significant. Software Review Manager (RevMan 5.2, Copenhagen) and STATA (Stata 11.2, Texas) were used for the statistical analysis.

RESULTS

Study selection

Figure 1 illustrates the process of study selection for the meta-analysis. Of the 1405

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potentially relevant references identified by electric and hand search, 142 were selected for full-text review after screening titles and abstracts. Finally, a total of 14 studies was included, with 3 RCTs,[21-23] 5 cohort studies,[24-28] and 6 case-control studies.[29-34] Of note, one of the case-control studies was presented solely in abstract form.[29] For the cohort study conducted by Friedman *et al.,*[25] in which the RR estimate were reported separately for different gender (male and female), these two reports were regarded as separate studies in our meta-analysis. Therefore, a total of fifteen reports were included for the present meta-analysis.

Study characteristics

Table 1 summarizes the characteristics of qualified studies in this meta-analysis. The 14 studies, involving 1,779,630 participants with 35,775 liver cancer cases, were published between 2005 and 2013. Except one RCT without identify information,[23] one cohort adopted ICD-10 C22,[24] all other studies identified liver cancer cases according to the ICD-9 155.

The three "RCTs" in the present study were pooled analyses of other RCTs (n=33),[21-23] which investigated statins therapy in cardiovascular event prevention and reported the incidence of liver cancer as adverse event. The observational studies were all conducted with the local or national health databases, the statin exposure were identified by linkage to prescription databases, and the controls were matched mainly by age, sex and index date. Two cohort studies were restricted to specified patients, such as patients with HBV infection,[27] or HCV infection.[28] One case-control studies was restricted to patients with diabetes mellitus.[30] Meanwhile, two observational

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studies were restricted to older patients.[26 31]

Table 2 summarizes the data provided by the included studies. In two RCT [22 23] and one pre-matched cohort study,[26] in which the RR estimates were not provided by the original studies, the RR with 95% CIs were calculated from the 2×2 tables defined by the incidence of liver cancer and the statin use status. Other studies reported different measurements of RR estimates with adjustment by potential confounders. Only several observational studies adopted at least 4 important risk factor for adjustments, such as HBV infection, HCV infection, cirrhosis, non-alcoholic fatty liver disease (NAFLD), HCV treatment, HBV treatment or anti-diabetic medications.[35] Meanwhile, only two studies adopted the cholesterol level for adjustments.[26 34]

Methodological quality

For the cohort and case-control studies, the median of Newcastle-Ottawa Scale scores was 7, with a range of 5 to 8 (**Supplementary Table 1**). These results indicated that the observational studies were in a reasonable good quality.

Overall meta-analysis

Figure 2 depicts the forest plot of RR estimate with 95% CIs from individual studies and overall meta-analysis. In the overall meta-analysis, pooled results showed a statistically significant decrease in the liver cancer risk among all statin users (RR 0.58, 95% CIs 0.51–0.67). Of note, a statistically significant heterogeneity was observed (I^2 = 59%, P=0.002). The P-values of Begg's test and Egger's test were 0.921 and 0.716, respectively, both suggesting there were no evidence of publication bias.

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Subgroup analyses

We first performed preplanned subgroup analyses of studies based on study design, study location confounding adjustment, and baseline risk of liver cancer. (**Table 3**).

In RCTs, only a non-significant decrease of liver cancer risk among all statin users was found (RR 0.95, 0.62–1.44; I^2=0%, P=0.59). Subgroup analyses of cohort studies found a greater decrease of liver cancer risk than the case-control studies among all statin users (RR 0.51, 0.44–0.58; I^2=18%, P=0.30 and RR 0.63, 0.54–0.73; I^2=46%, P=0.10, respectively). Test for subgroup differences (I^2=79.9%, P=0.007) indicated the study design partly explained the heterogeneity in the overall analysis. (**Figure 2**)

Subgroup analysis of studies with higher baseline risk of liver cancer,[26-28 31] defined as older patients, HBV or HCV infected patients, found a greater decrease of liver cancer risk (RR 0.52, 0.47-0.59; I^2=16%, P=0.31) than the studies with general population and other population (RR 0.60, 0.49–0.75; I^2 = 48%, P=0.05 and RR 0.72, 0.62–0.83; I^2 =0%, P=0.34, respectively). Test for subgroup differences (I^2=82.7%, P=0.003) indicated that the difference in baseline risk of liver cancer can partly explained the heterogeneity in the overall analysis (**Supplementary Figure 1**).

Subgroup analysis of studies that adjusted adequately,[21-23 29-32] which defined as RCTs or adjusted for at least 4 of 7 important confounders, found a less decrease of liver cancer risk among all statin users (RR 0.67, 0.55-0.83; I^2=47% P=0.09). Subgroup analysis of studies that adjusted inadequately found a greater decrease of liver cancer risk among all statin users (RR, 0.54, 0.47-0.62; I^2=40%, P=0.10).[24-28 33 34] Test for subgroup differences (I^2=69.2%, P=0.07) indicated the confounding adjustment also

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partly explained the heterogeneity in the overall analysis (**Supplementary Figure 2**).

Then, we conducted subgroup analyses based on studies use lipophilic statins and higher cumulative dosage of statin, when appropriate data were available.

Subgroup analysis based on study location found similar results in Western countries and Asian countries (RR 0.61, 0.49–0.76; I^2 = 59%, P=0.01 and RR 0.55, 0.49–0.63; I^2 =26%, P=0.23, respectively). Test for subgroup differences (I^2 =0%, P=0.47) found no significant heterogeneity between these two subgroups (**Supplementary Figure 3**).

Subgroup analysis of studies with use of lipophilic statins, such as atorvastatin, fluvastatin, lovastatin, and simvastatin, was conducted based on the pharmacokinetic data.[23 25 27 30-32] The pooled results indicated a significant decrease of liver cancer risk among users of lipophilic statins (RR 0.57, 0.50–0.65; I^2=40%, P=0.13). (**Supplementary Figure 4**)

Subgroup analysis of studies with higher cumulative dosage of statin use, defined as cumulative defined daily dose (cDDDs) > 180 or cumulative duration of statin use > 0.5 years, also found a significant decrease of liver cancer risk (RR 0.54, 0.38-0.77), but with a high degree of degree of heterogeneity (I^2=85%, P<0.00001). (**Supplementary Figure 5**)

DISCUSSION

This present meta-analysis represents the most comprehensive review to date on the relation between the statin use and the liver cancer risk, by including 14 studies (3 RCTs, 5 cohort studies, and 6 case-control studies) and involving 1,779,630

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participants with 35,775 liver cancer cases. Overall, we found a significant inverse association between statin use and risk of liver cancer (RR 0.58, 95% CIs 0.51–0.67), when statins were taken at daily doses for cardiovascular event prevention. This result was in line with the previous three meta-analyses that only included some of our included studies: Singh *et al.* included 10 studies and suggested statin users were less likely to develop HCC than statin nonusers (Odds Ratios 0.63, 95% CIs 0.52-0.76),[16] Pradelli *et al.* and Zhang *et al.* included 5 and 7 observational studies and found a summary RR of 0.58 (95% CIs 0.46–0.74) and 0.61 (95% CIs 0.49–0.76), respectively. [36 37]

The inverse association between the statin use and the liver cancer risk was seen primarily in observational studies, and which was relative stronger in the cohort studies than the case-control studies. Subgroup analysis of RCTs only found a non-significant inverse association, mainly because of the RCTs included low risk population (Cardiovascular disease patients rather than HBV /HCV infected patients). Meanwhile, subgroup analysis of studies with higher baseline risk of liver cancer, found a greater decrease of liver cancer risk than the studies with general population and other population. These results all indicated that the protective effect of statins might vary according to different baseline risk.

Subgroup analysis of adjusted adequately studies found a less decrease of liver cancer risk than the adjusted inadequately studies, indicated the potential of overestimate the preventive effect of statins by inadequately adjustment. On the other hand, there were inverse association between use of non-statin lipid-lowering drugs and risk of the liver

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cancer.[31 34] Meanwhile, some clinical studies demonstrated that higher serum total cholesterol concentration was associated with decreased risk of liver cancer (**Supplementary Table 3**).[38-40] Unfortunately, the studies we included seldom adopt these two factors for adjustment. These fact all indicated that the statin indication (e.g. hyperlipidemia) might overestimate its chemopreventive effect.

We found similar results in Western countries and Asian countries, which were different from the meta-analysis conducted by Singh *et al.* which concluded that the inverse association of statins with HCC was stronger in the Asian population. Considering we included four more studies, this difference might be caused by the insufficient data in their meta-analysis.

The lipophilic properties of the statins are accompanied by an extensive first-pass effect at the hepatic level.[41] It is plausible that lipophilic statins will differ in their liver cancer prevention qualities.[42] However, subgroup analysis of studies with lipophilic statins found similar results with a summary RR of 0.57 (95% CIs 0.50-0.65). In our study, there was a trend toward more reduction of liver cancer risk with higher cumulative dosage of statin use (RR 0.54, 95% CIs 0.38–0.77), which showed the potential of dose-response relationship.

Besides the previously described limitations, there were several other limitations should be noted when interpreting our findings. First, a significant heterogeneity was observed in the present meta-analysis (I^2 = 59%, P=0.002), and the difference in study design, baseline risk and confounding adjustment might explained the significant heterogeneity. Results of other subgroup analyses, which pooling the data all studies

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together, would also be limited by this heterogeneity. Second, other factors may affect the estimate of RR for liver cancer. For example, the adherence to statin therapy is known to be associated with healthy lifestyle, which might affect the cancer outcome.[43] Such information is hard to be captured in databases or medical record in the observational studies.[44] Third, five observational studies were conducted using the Taiwanese National Health Insurance Research Database (NHIRD),[27 28 31-33] though they were not in the same period, there was still a potential that these studies contained overlapping groups of patients. Although the confounding factors mentioned above may have a limited effect on our overall results from the present study, these factors should be considered in future studies aiming at confirming the protective effects of statins on human cancer risk.

The strengths of our meta-analysis were as follows: First, we performed a much more comprehensively search and more subgroup analyses, compared with the previous meta-analyses; Second, the methodological quality of the included studies was reasonable good; Third, publication bias, which due to the tendency of not publishing small studies with null results, were not found in our meta-analysis.

Currently, physicians are less likely to prescribe statins for patients with chronic liver disease, which are known risk factors of liver cancer, based on the concerns about the statin-induced liver injury.[27] However, there were number of studies have demonstrated the safe use, even salutary effects, of statins in patients with HCV infection, HBV infection or NAFLD.[27 28 45-47] Meanwhile, the risk of serious statin-related liver injury appears to be no greater than the background incidence of

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this rare event.[48] Therefore, considering their benefits for cardiovascular event prevention and potential in liver cancer prevention, statins should not be denied to the patients with chronic liver diseases.

Of note, preclinical studies have indicated that statins possess synergism with other therapeutic agents *in vitro* and *in vivo* for liver cancer.[49 50] Meanwhile, clinical studies have also demonstrated that statins would prolong survival in patients with advanced liver cancer (**Supplementary Table 4**).[51-54] Moreover, statin use might associate with decrease of cancer recurrence risk in patients of HBV related HCC after curative surgery.[55] Therefore, considerable interest exists in adjunctive therapy with statins for liver cancer. In fact, there were several prospective, randomized, controlled trials ongoing to determine the effectiveness of pravastatin in the treatment of liver cancer, when used in combination with sorafenib (**Supplementary Table 5**).

In conclusion, our results suggest there is a significant inverse association between the statin use and the risk of liver cancer, when statins are taken daily for cardiovascular event prevention. However, some confounders might overestimate the preventive effect. Further studies are needed to investigate the efficacy of statins in the prevention and treatment of liver cancer.

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FIGURE LEGENDS:

Figure 1. Flow chart of study selection in the present meta-analysis.

Figure 2. Overall meta-analysis of the statin use and the liver cancer risk.

Supplementary Figure 1. Subgroup analyses based on baseline risk of liver cancer.

Supplementary Figure 2. Subgroup analysis based on confounding adjustment.

Supplementary Figure 3. Subgroup analysis based on study location.

Supplementary Figure 4. Subgroup analyses of use of lipophilic statins.

Supplementary Figure 5. Subgroup analyses of higher cumulative dosage of statin use.

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Table 1. Study characteristics

Studies	Study design	Patient population	Study period	Cases defined	Follow-up	Statins type	Dosage/Duration of Statin use
Stein, 2006, USA [23]	RCT	Pooled analysis of 8 RCTs	-	NR	2.4 years (M)	F, 20–80 mg daily	2.4 years (M)
Matsushita, 2010, Japan [21]	RCT	IPD analysis of 3 RCTs	-	ICD-9 155	≥4.7 years	P, 10-20 mg daily	≥4.7 years
Emberson, 2012, UK [22]	RCT	IPD analysis of 22 RCTs	-	ICD-9 155	5.1 years (Me)	A, F, L, P, R, S	5.1 years (Me)
Friis, 2005, North Jutland [24]	Cohort	General population (CPR)	1989-2002	ICD-10 C22	3.3 years (M)	Unspecified	≥2 Rx
Friedman, 2008, USA [25]	Cohort	General population (KPMCP)	1994-2003	ICD-9-CM 155	> 2 years	A, L, S (97.6%)	≥1 Rx
Marelli, 2011, USA [26]	Cohort	General older population (men ≥ 45 and women ≥ 55 years; GE Centricity)	1990-2009	ICD-9 155	4.6 years (M)	Unspecified	≥1 cDDD
Tsan, 2012, Taiwan [27]	Cohort	Patients with HBV infection (NHIRD)	1997-2008	ICD-9 155	9.9 years (M)	A, F, L, P, R, and S	≥28 cDDDs
Tsan, 2013, Taiwan [28]	Cohort	Patients with HCV infection (NHIRD)	1999-2010	ICD-9 155	10.7 years (M)	A, F, L, P, R, and S	≥28 cDDDs
Khurana, 2005, USA [29]	Case control	General population (VISN)	1997-2002	ICD-9 155	NR	Unspecified	≥1 Rx
El-Serag, 2009, USA [30]	Case control	Diabetes patients (VA)	1997-2002	ICD-9-CM 155	2.4 years (M)	A, C, F, L, P, and S	1.6 years (M)
Chiu, 2011, Taiwan [31]	Case control	Older patients（≥ 50 years; NHIRD）	2005–2008	ICD-9-CM 155	NR	A, F, L, P, R, and S	≥ 1 cDDD
Lai, 2013, Taiwan [32]	Case control	General population (NHIRD)	2000-2009	ICD-9-CM 155	1.4 years (M)	A, F, L, P, R, and S	≥1 Rx
Leung, 2013, Taiwan [33]	Case control	General population (NHIRD)	2000-2008	ICD-9-CM 155	4.1 years (M)	Unspecified	> 0.5 years
Chaiteerakij, 2013, USA [34]	Case control	Hyperlipidemia patients (Mayo Clinic)	2000-2010	ICD-9-CM 155	>1 years	Unspecified	≥1 Rx

Patients population: IPD=Individual patient data, RCT = randomized controlled trials, CRP=the Central Population Register of Danish citizens, KPMCP=the Kaiser Permanente Medical Care Program in northern California, GE Centricity=the General Electric Centricity database, NHIRD=the Taiwanese National Health Insurance research database, VISN=Veterans Integrated Service Networks 16 Veteran Affairs database, VA=Veterans Affairs national databases, Mayo Clinic= Mayo Clinic (Rochester, MN), HBV = hepatitis B virus; Cases defined: ICD-9 or -10 =International Classification of Diseases, Ninth Revision or Tenth Revision, CM=Clinical Modification; Duration of follow-up: When the follow-up periods of statin user and nonuser were different, only the shorter one was showed, and all periods were transformed to years; Statin type: A=Atorvastatin, C=Cerivastatin, F=Fluvastatin, L=Lovastatin, P=Pravastatin, R=Rosuvastatin, S=Simvastatin, Non-statin= Non-statin cholesterol-lowering drug(s) only; Duration of statin use: M=Mean, Me=Median, ≥1 cDDD = more than 1 cumulative defined daily dose before the diagnosis of liver cancer, Rx=prescriptions.

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Table 2. Study data

Studies	Intervention/ Cases		Control		Measurements of effect estimates	Crude RR with 95% CIs	Adjusted RR with 95% CIs	Confounders for adjustment
	No. of event/ No. of exposure	No. of total	No. of event/ No. of exposure	No. of total				
Stein, 2006, USA [23]	3	3512	4	3289	RR	0.70 (0.16-3.14)*	0.70 (0.16-3.14)*	Randomization
Matsushita, 2010, Japan [21]	5	7375	7	6349	HR	NA	0.58 (0.18-1.84)	Randomization
Emberson, 2012, UK [22]	35	67258	33	67279	RR	1.06 (0.66, 1.71)*	1.06 (0.66, 1.71)*	Randomization
Friis, 2005, North Jutland [24]	1	12251	166	334754	OR	NA	1.16 (0.46-2.90)	1,2, 16, 21, 23
Friedman(Male), 2008, USA [25]	32	192598	NA	NA	HR	NA	0.49 (0.34-0.70)	16
Friedman(Female), 2008, USA [25]	10	169261	NA	NA	HR	NA	0.40 (0.21-0.75)	
Marelli, 2011, USA [26]	13	45857	24	45857	RR	0.31 (0.14-0.68)*	0.31 (0.14-0.68)*	1-5, 14, 16-18, 26, 27
Tsan, 2012, Taiwan [27]	58	2785	963	30628	HR	0.66 (0.51- 0.86)	0.47 (0.36-0.61)	1, 2, 7, 8, 11, 12
Tsan, 2013, Taiwan [28]	1378	35023	26505	225841	HR	0.42 (0.39-0.46)	0.53 (0.49–0.58)	1, 2, 7, 8, 11, 13
Khurana, 2005, USA [29]	NA	NA	NA	NA	OR	NA	0.52 (0.41- 0.67)	1, 11, 13
El-Serag, 2009, USA [30]	447	1303	2766	5212	OR	0.46 (0.40-0.52)	0.74 (0.64-0.87)	1-3, 6, 8, 9, 11-13, 21, 24, 28
Chiu, 2011, Taiwan [31]	117	1166	195	1166	OR	0.53 (0.41-0.69)	0.62 (0.45-0.83)	1, 2, 8, 9, 11, 12, 20, 29
Lai, 2013, Taiwan [32]	255	3480	1635	13920	OR	0.61 (0.52–0.72)	0.71 (0.56–0.89)	1, 2, 8-13, 22, 24, 25
Leung, 2013, Taiwan [33]	26	424	6851	33781	HR	0.45 (0.30-0.67)	0.44 (0.28, 0.72)	1, 2, 11, 15, 20, 21, 23
Chaiteerakij, 2013, USA [34]	72	165	165	256	OR	NA	0.6 (0.4-0.9)	1-3, 8, 11, 17, 22, 28, 30

*=the RR was calculated based on raw data; Adjusted RR=RR adjusted for confounders; Confounders for adjustment: 1=age, 2=sex, 3=race, 4=BMI, 5=smoking status, 6=ethanol intake, 7=socioeconomic status, 8=cirrhosis, 9=alcoholic liver disease, 10=non-alcoholic fatty liver disease, 11=diabetes mellitus, 12=HBV infection, 13=HCV infection, 14=concomitant diagnoses (unspecified), 15=Charlson score, 16=calendar year, 17=cholesterol (total cholesterol, VLDL, LDL, or triglycerides), 18=prostate-specific antigen, 19=resection extent, 20=other lipid-lowering agents, 21=cardiovascular medications (aspirin, nonsteroidal anti-inflammatory medications, or angiotensin-converting enzymes inhibitors), 22=metformin or thiazolidinedione, 23=hormone-replacement therapy, 24=HCV treatment, 25=HBV treatment, 26=medications taken (unspecified), 27=the number of office visits, 28=propensity to use statins, 29=hospital stay, 30=biliary tract diseases

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Table 3. Subgroup analyses of included studies

Subgroup		No. of studies (reports)	Summary RR (95% CIs)	Heterogeneity, I^2	Heterogeneity, *P* value	Test for subgroup differences, I^2	Test for subgroup differences, *P* value
Study design	RCTs	3	0.95 (0.62-1.44)	0%	*P*=0.59	79.9%	***P*=0.007**
	Cohort studies	5 (6)	0.51 (0.44–0.58)	18%	*P*=0.30		
	Case-control studies	6	0.63 (0.54–0.73)	46%	*P*=0.10		
Baseline risk of liver cancer	Higher baseline risk	4	0.52 (0.47, 0.59)	16%	*P*=0.31	82.7%	***P*=0.003**
	General population	8 (9)	0.60 (0.49–0.75)	48%	*P*=0.05		
	Other population	2	0.72 (0.62–0.83)	0%	*P*=0.34		
Confounding adjustment	Adjusted adequately studies	6	0.67 (0.56-0.83);	47%	*P*=0.09	69.2%	***P*=0.07**
	Adjusted inadequately studies	8 (9)	0.58 (0.51-0.66)	40%	*P*=0.10		
Study location	Western studies	8 (9)	0.61 (0.49-0.76)	59%	*P*=0.01	0%	*P*=0.49
	Asian studies	6	0.56 (0.49- 0.64)	38%	*P*=0.15		
Use of lipophilic statins		6 (7)	0.57 (0.50-0.65)	40%	*P*=0.13	-	-
Higher cumulative dosage of statin		8	0.54 (0.38- 0.77)	85%	*P*<0.0001	-	-

RR= relative risk; higher baseline risk: older patients, HBV or HCV infected patients. Confounding adjustment: Adjusted adequately means reported RR have been adjusted for at least 4 of 7 important factors: HBV infection, HCV infection, cirrhosis, NAFLD, HCV treatment, HBV treatment, Anti diabetic medications; Lipophilic statin use: use of atorvastatin, fluvastatin, lovastatin, or simvastatin; Higher cumulative dosage of statin use: > 180cumulative defined daily dose or Duration of statin use > 0.5 years before the diagnosis of liver cancer.

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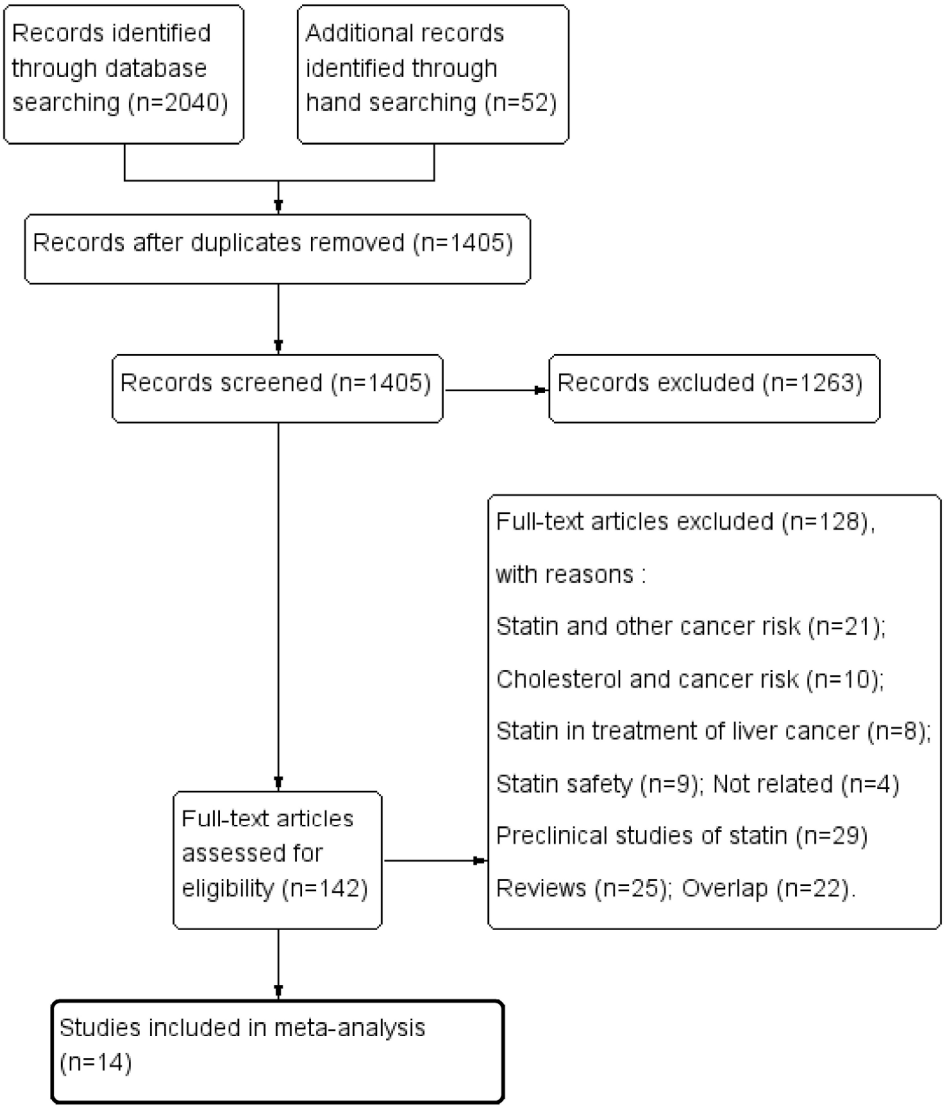

Flow chart of study selection in the present meta-analysis.
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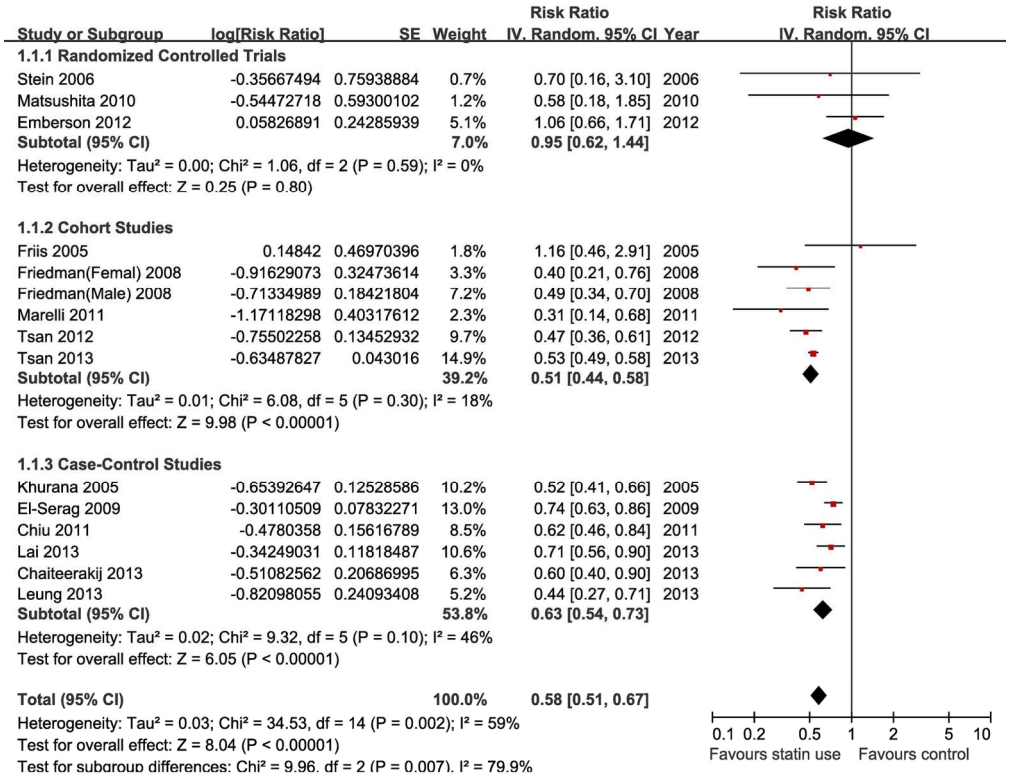

Overall meta-analysis of the statin use and the liver cancer risk
152x118mm (300 x 300 DPI)

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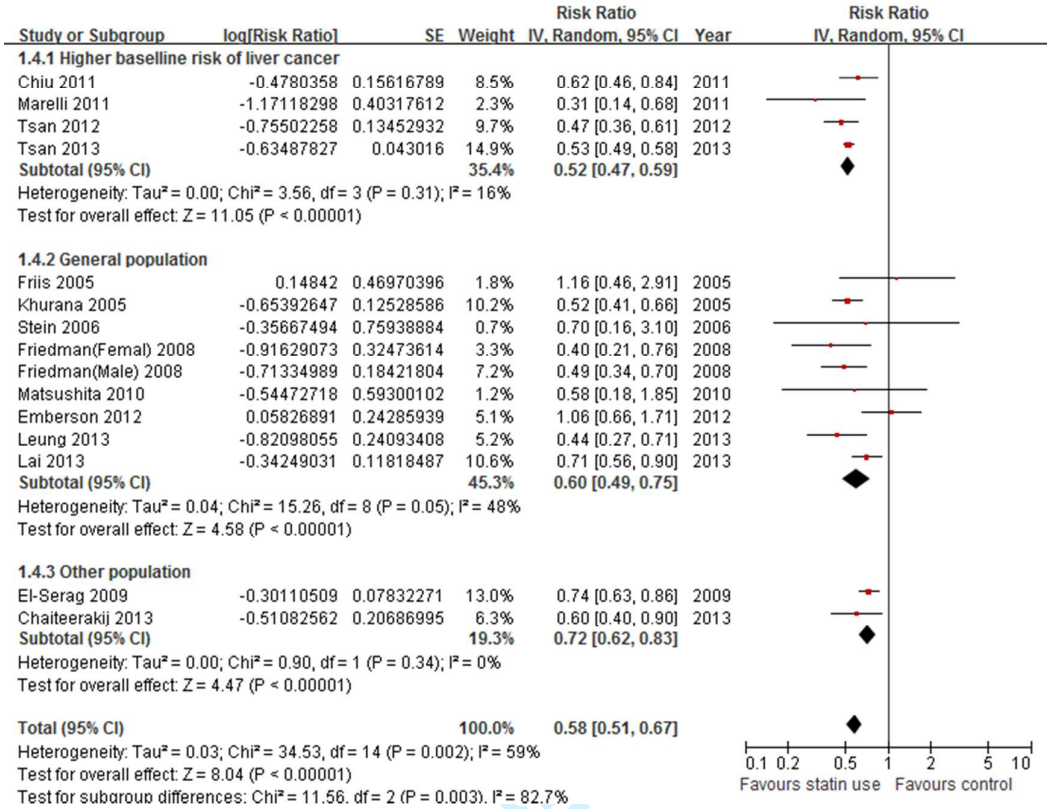
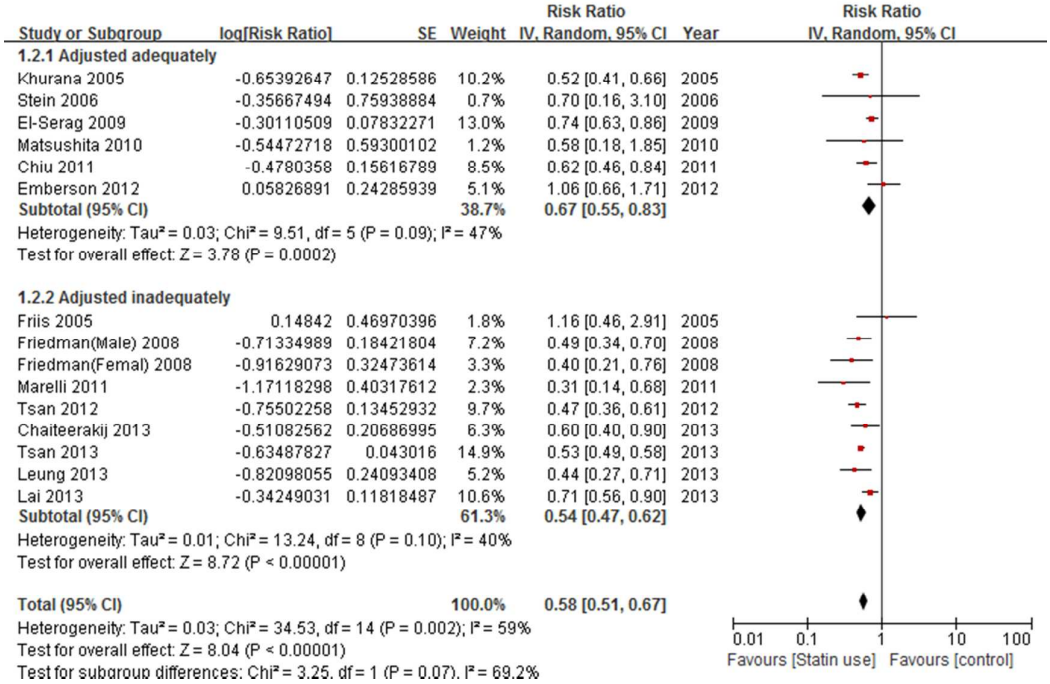
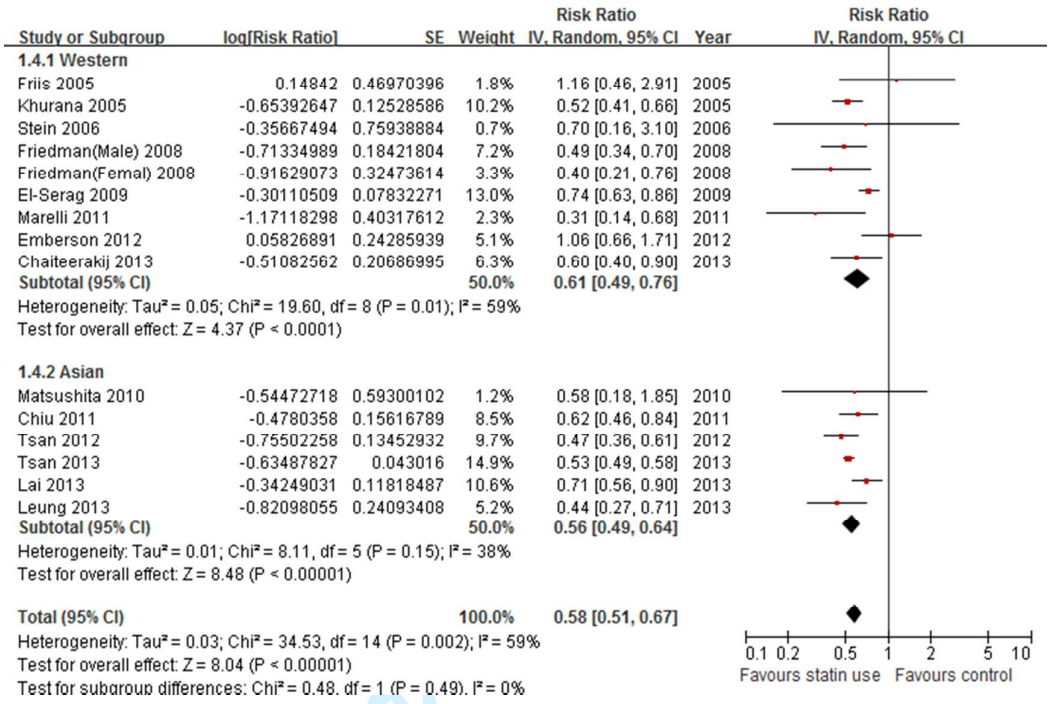
SUPPLEMENTARY FIGURES:



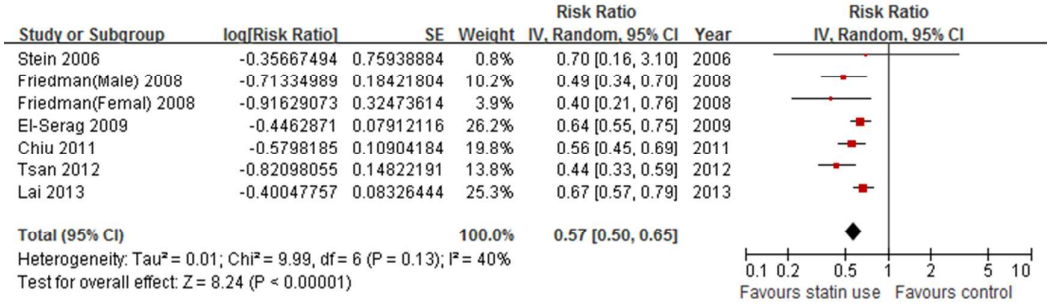
Supplementary Figure 1. Subgroup analyses based on baseline risk of liver cancer.



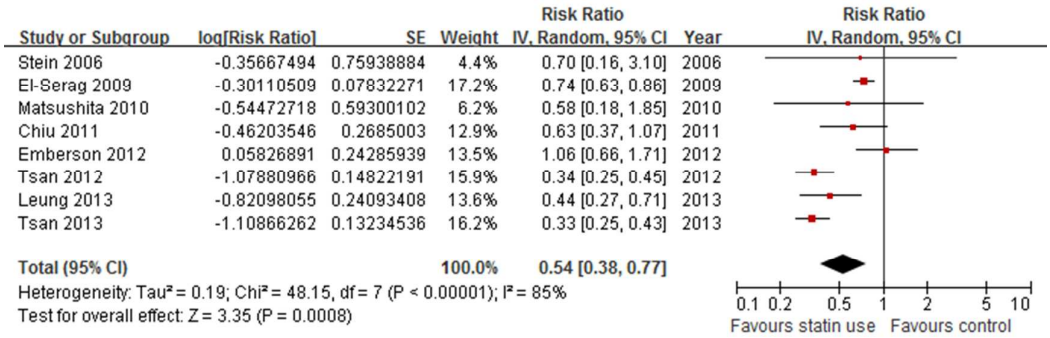
Supplementary Figure 2. Subgroup analysis based on confounding adjustment.



Supplementary Figure 3. Subgroup analysis based on study location.



Supplementary Figure 4. Subgroup analyses of use of lipophilic statins.



Supplementary Figure 5. Subgroup analyses of higher cumulative dosage of statin use.

SUPPLEMENTARY TABLES:

Supplementary Table 1. Assessment of methodological quality of the cohort and case-control studies according to the Newcastle–Ottawa Scale

Cohort Studies	Selection				Comparability		Outcome		Total Score
	Representativeness of the exposed cohort	Selection of the non-exposed cohort	Ascertainment of exposure	Outcome of present at start of study	Control for important factor	Assessment of outcome	Follow-up long enough	Adequacy of follow up	
Friis, 2005 [24]	★	★	★	★	★	★	-	★	7
Friedman, 2008 [25]	★	★	★	★	★	★	-	★	7
Marelli, 2011 [26]	★	★	★	★	★	★	★	★	8
Tsan, 2012 [27]	★	★	★	★	★	★	★	★	8
Tsan, 2013 [28]	★	★	★	★	★	★	★	★	8
Case–Control Studies	**Selection**				**Comparability**		**Exposure**		**Total Score**
	Adequate definition of cases	Representativeness of cases	Selection of controls	Definition of controls	Control for important factor	Ascertainment of Exposure	Same method for cases and controls	Non-response rate	
Khurana, 2005 [29]	-	★	★	★	★	★	★	-	6
El-Serag, 2009 [30]	-	★	★	★	★★	★	★	-	7
Chiu, 2011 [31]	-	★	★	★	★★	★	★	-	7
Lai, 2013 [32]	-	★	★	★	★★	★	★	-	7
Leung, 2013 [33]	★	★	★	★	★	★	★	★	8
Chaiteerakij, 2013 [34]	-	★	-	★	★	★	★	-	5

Control for important factor: ★ Reported relative risk have been adjusted for at least 4 of 7 important factors: HBV infection, HCV infection, cirrhosis, NAFLD, HCV treatment, HBV treatment, anti-diabetic medications; ★ Study controls for any additional factor. Assessment of outcome: ★ record linkage. Follow-up long enough: ★ follow up period ≥ 4 years. Adequate definition of cases: ★ The case is defined with independent validation. Non-response rate: ★ Same rate for both groups.

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Supplementary Table 2. Studies reporting RR for use of lipophilic statins and for higher cumulative dosage of statin use

Studies	Measurements of effect estimates	Statins type	Dosage/Duration of Statin use	Crude RR with 95% CIs	Adjusted RR with 95% CIs
Tsan, 2012, Taiwan [27]	HR	A, F, L, P, R, and S	>365 cDDDs	0.50 (0.26-0.96)	0.34 (0.33-0.59)
	HR	Lipophilia statin	≥28 cDDDs	0.65 (0.39 -1.09)	0.44 (0.33-0.59)
Tsan, 2013, Taiwan [28]	HR	A, F, L, P, R, and S	>180 cDDDs	NA	0.33 (0.25–0.42)
El-Serag, 2009, USA [30]	OR	Simvastatin	1.6 years (M)	0.47 (0.41- 0.54)	0.64 (0.55-0.75)
Chiu, 2011, Taiwan [31]	OR	A, F, L, P, R, and S	>215.4 cDDDs	0.47 (0.30-0.72)	0.63 (0.37-1.06)
	OR	Lipophilia statin	≥ 1 cDDD	0.56 (0.45–0.69)*	0.56 (0.45–0.69)*
Lai, 2013, Taiwan [32]	OR	Lipophilia statin	≥1 Rx	0.67 (0.57–0.79)*	0.67 (0.57–0.79)*

*= RR was calculated based on raw data; Adjusted RR=RR adjusted for confounders

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Supplementary Table 3. Published studies of the total cholesterol and the risk of liver cancer

Studies	Study design	cases/ participants	Follow-up	Reference (mg/dL)	Index (mg/dL)	Adjusted HR (95% CIs)		*P* for trend*	Confounders for adjustment
						Men	Women		
Iso, 2009, Japan [39]	Population-based cohort (JPHC Study)	125 /33,368	12.4 years	180–199	< 160	2.62 (1.44–4.76)	4.15 (1.70–10.16)	Men < 0.0001 Women < 0.0001	1-10
					160–179	1.04 (0.52–2.07)	1.99 (0.82–4.85)		
					180–199	1	1		
					200–219	0.56 (0.24–1.28)	1.09 (0.44–2.68)		
					200–239	0.49 (0.16–1.44)	0.41 (0.11–1.52)		
					> 240	-	0.80 (0.28–2.27)		
Ahn, 2009, Finland [38]	Placebo-controlled, double-blinded primary prevention trial in male smokers (ATBC)	191/29,093	18.0 years	< 203.9	< 203.9	1	-	P=0.0007	1-5, 11-17
					203.9-227.6	0.69 (0.46-1.05)	-		
					227.7-249.2	0.63 (0.41-0.97)	-		
					249.3-276.6	0.56 (0.36-0.88)	-		
					> 276.7	0.66 (0.43-1.01)	-		
Kitahara, 2011, Korea [40]	Prospective study of Korean men and women (Korean NHIC)	10,161/1,189,719	12.7 years	< 160	< 160	1	-	Men < 0.001 Women < 0.001	2-5, 13, 18
					160-179	0.69 (0.65-74)	0.63 (0.54-0.72)		
					180-199	0.62 (0.58-0.66)	0.50 (0.44-0.58)		
					200-239	0.48 (0.45-0.51)	0.37(0.32-0.42)		
					≥ 240	0.42 (0.38-0.45)	0.32 (0.27-0.39)		

JPHC Study= The Japan Public Health Center-based Prospective Study，ATBC=The Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study, Korean NHIC= The Korean National Health Insurance Corporation Medical Evaluation. *Tests for linear trend were conducted by treating the total cholesterol as a continuous variable in the multivariable models. **Confounders for adjustment**: 1=age, 2=BMI, 3=smoking, 4=ethanol intake, 5= hypertension, 6=diabetes, 7=hyperlipidemia medication use, 8=total vegetable intake, 9=coffee Intake, 10=public health center, 11=intervention, 12=level of education, 13=physical activity, 14=Saturates fat intake, 15=polyunsaturated fat intake, 16=total calorie, 17=serum HDL cholesterol, 18=fasting serum glucose.

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Supplementary Table 4. Published trials of statin use in treatment of liver cancer

Studies	Study design	Patients population	Intervention	Control	Overall survival of intervention (months)	Overall survival of control (months)	Kaplan-Meier and log-rank test
Kawata, 2001, Japan [51]	Prospective, randomized, open label study	Patients with advanced liver cancer after TAE procedure, n=83	Pravastatin 20-40 mg + 5-FU 200 mg QD, n=41	5-FU 200 mg QD, n=42	Median 18	Median 9	$P = 0.006$
Lersch, 2004, Germany [52]	Prospective study	Patients with advanced liver cancer, n=58	Pravastatin 40-80 mg QD, n=20	A: Octreotide, n=30; B: Gemcitabine, n=8	Median 7.2 (95% CIs 2.9-11.5)	A: Median 5 (95% CIs 2.2-7.8); B: Median 3.5 (95% CIs 2.2-4.9)	A: $P = 0.09$; B: $P = 0.03$
Graf, 2008, Germany [53]	Prospective, non-randomized, open label study	Patients with advanced liver cancer after TACE, n=183	Pravastatin 20-40 mg QD, n=52	No treatment, n=131	Median 20.9 (95% CIs 15.5-26.3)	Median 20.9 (95% CIs 15.5-26.3)	$P = 0.003$
Georgescu, 2011, Romania [54]	Prospective, randomized, open label study	Patients with advanced liver cancer, n=72	Lovastatin 40 mg + Sorafenib 400 mg QD, n=39	Sorafenib 400 mg QD, n=33	Mean 12.15±0.76	Mean 10.85±0.82	Non-significant

TAE = Transcatheter arterial embolization; TACE = Transarterial chemoembolization.

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Supplementary Table 5. Ongoing clinical trials of statin use in treatment of liver cancer

Studies	Year	Location	Phase	Study design	Condition	Intervention	Control	Estimated Enrollment	Resist number	Status
ESTAHEP-2010	2011	Spain	II	Multicenter, prospective, randomized, double-blind, placebo-controlled study	Advanced liver cancer	Sorafenib 400 mg BID + Pravastatin 40 mg, QD	Sorafenib 400 mg BID + placebo QD	216	NCT01418729; EUCTR2010-024421-21-ES	Recruiting
PRODIGE 21	2011	France	II	Multicenter, prospective, randomized, open label study	Liver cancer with Child-Pugh B Cirrhosis	A: Sorafenib 400 mg BID; B: Pravastatin 40 mg, QD; C: Sorafenib 400 mg BID + Pravastatin 40 mg, QD	Best supportive care	160	NCT01357486	Recruiting
JOUVE PHRCK 2009	2013	France	III	prospective, randomized, open label study	Liver cancer with Child-Pugh A Cirrhosis	Sorafenib 800 mg BID + Pravastatin 40 mg, QD	Sorafenib 800 mg BID	474	NCT01903694; NCT01075555	Recruiting

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PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	3
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	4
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	no
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	4-5
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	4
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	4
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	4
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	5
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	5
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	5-6
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	6
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	6

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PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	6
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	6
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	7
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	7, 19, 20
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	8, Suppl. Table 1
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	Figure 2
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	8
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	8
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	8-10
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	10-11
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	11-13
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	14
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	NA

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Statin use and risk of liver cancer: an update meta-analysis

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Running title: Meta-analysis: statin and liver cancer

Key words: *Statin; Liver cancer; Cancer Prevention; Meta-analysis.*

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Abstract

Objective: Statins are commonly prescribed cholesterol-lowering drugs. Preclinical studies suggest that statins may possess cancer preventive properties. The primary objective of this meta-analysis was to determine the association between the statin use and the risk of liver cancer.

Design: Meta-analysis.

Setting: International.

Participants: A comprehensive literature search of PubMed, BIOSIS Previews, Web of Science, EMBASE, EBSCO and Cochrane Library was conducted through March 2014. The effect estimate was reported as pooled relative risk (RR) with 95% confidence intervals (CIs), using the random-effects model.

Results: A total of 12 studies (one individual patient data analysis of 22 randomized controlled trials, 5 cohorts, and 6 case-controls) were qualified for this meta-analysis, involving 5,640,313 participants including 35,756 liver cancer cases. Our results indicated a significant risk reduction of liver cancer among all statin users (RR 0.58, 95%CIs 0.51–0.67). The difference of the study designs can partly explained the significant heterogeneity found in the overall analysis (I^2 = 65%, P = 0.0006). No evidence of publication bias was observed in this meta-analysis. Similar risk reductions were found in the subgroups analysis of Western and Asian countries, lipophilic and hydrophilia statins. There was a trend toward more risk reductions in subgroups with higher baseline risk, inadequate adjustment, and higher cumulative dosage of statin use.

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Conclusions: This meta-analysis suggests that the statin is associated with a significant risk reduction of liver cancer, when taken daily for cardiovascular event prevention. However, this preventive effect might be overestimated due to the exposure period, tthe indication and contraindication of statins, and other confounders. Statins might be considered as an adjuvant in the treatment of liver cancer.

Strengths and limitations of this study

Statins are commonly prescribed as cholesterol-lowering drugs. In this comprehensive meta-analysis, we demonstrate that the statin use is associated with a significant risk reduction of liver cancer.

The difference of the study designs is the part reason that explained the significant heterogeneity found in the overall analysis.

However, this preventive effect might be overestimated due to the exposure period, tthe indication and contraindication of statins, and other confounders.

Statins might be considered as an adjuvant in the treatment of liver cancer.

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Introduction

Statins are inhibitors of 3-hydroxy-3-methylglutaryl coenzyme-A (HMG-CoA) reductase and they are widely used to reduce the plasma cholesterol level and the risk of cardiovascular events.[1] Although there is a concern over their possible carcinogenicity raised in rodent studies,[2] preclinical studies indicate that statins have anticancer properties *in vitro* and *in vivo*, through inhibiting angiogenesis, inducing apoptosis, and suppressing tumor growth and metastasis.[3-5]

However, higher concentrations of statins are typically required to induce these effects, raising questions concerning the therapeutic relevance of statins on cancer.[6] To date, clinical studies regarding the cancer incidence associated with statin administration have highlighted conflicting results. Moreover, a large number of meta-analyses have concluded that there was no association between statin use and risk of overall cancer,[7-10] or cancer of breast[11], stomach,[12] or pancreas.[13] There is only a modest protective effect of statins in prostate cancer[14] and colorectal cancer.[15]

In contrary, recent studies reported encouraging results for risk reduction of liver cancer among all statin users. Previous meta-analysis, conducted by Singh *et al*. by including 10 studies, found that statin users were less likely to develop hepatocellular carcinoma (HCC) than statin non-users.[16] However, Singh *et al*. included the ALERT, LIPS, and MEGA trials twice, by including three individual patient data (IPD) analysis of randomized controlled trials (RCTs).[17-19] Meanwhile, some factors of stratification were not considered in their analyses, such as dose and timing of exposure to statins, and the selection of controls and confounders, which might limit

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the evaluation of cancer risk.[20] Furthermore, the lipophilic statins are accompanied by an extensive first-pass effect at the hepatic level.[21] It is plausible that lipophilic statins may have a better liver cancer preventive qualities than the hydrophilic ones.[22]

Therefore, we performed this updated meta-analysis to assess the association between the statin use and the risk of liver cancer, involving the recently published studies and conducting more subgroup analyses based on the factors mentioned above. Our results demonstrated that statin use was associated with an over 40% risk reduction in liver cancer, which may have a significant translational potential in the clinical practice. However, there were some confounders might overestimate this preventive effect of statins.

MATERIALS AND METHODS

Literature Search strategy

This meta-analysis was conducted following the PRISMA guidelines.[23]

The systematic computerized search for eligible studies were performed on the database of PubMed, BIOSIS Previews, Web of Science, EMBASE, EBSCO, and Cochrane Library, covering all studies published from their inception to March 5, 2014. The following terms were searched with both the subjects (MeSH terms) and text-word search strategies: "(Statin OR HMG-CoA reductase inhibitors OR Atorvastatin OR Cerivastatin OR Fluvastatin OR Lovastatin OR Pravastatin OR Rosuvastatin OR Simvastatin) AND (Hepatocellular OR Hepatic OR Intrahepatic OR Interlobular OR Liver) AND (Carcinoma OR Sarcomas OR Angiosarcoma OR Cancer OR Neoplasm). Additionally, the relevant reviews and retrieved articles were searched

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manually for more eligible studies.

In study searching, only the original researches, published in form of peer review article or meeting abstract, were included. No language restrictions were imposed. However, the studies we included were all published in English.

Study selection

The inclusion criteria were: (1) randomized controlled trial (RCTs), cohort studies or case-control studies; (2) original studies that assessed the effect of statin use on the risk of liver cancer, compared with placebo or no treatment; (3) liver cancer cases were identified according to the International Classification of Diseases codes (ICD); and (4) studies with estimate of relative risk (risk ratio, RR) of liver cancer, or with data sufficient to calculate it.

The exclusion criteria were: (1) study design not meeting the inclusion criteria; (2) studies without estimate of RR, or without sufficient data to calculate it; or (3) studies with duplicated or overlap reports.

Data extraction

Two independent investigators (M. Shi and X.B. Cui) extracted data from the eligible studies using a predefined data collection form. The differences of data extraction were resolved by consensus referring back to the original article. The extracted information included: (1) Studies: first author, year of publication, study design, location, patient populations, period, and follow-up; (2) Statins: type, dosage or duration of statin use; (3) liver cancer: case identification, number of liver cancer, crude RR with 95% confidence intervals (CIs), adjusted RR reflecting the greatest

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degree of control for confounders, and confounders for adjustment (including variables for matching). When the RR were not available, the RR with 95% CIs were calculated from the raw data in original studies.

We extracted different measurements of effect estimates from original studies, such as Relative Risk (RR), Odds Ratio (OR), Hazard Ratio (HR), and Observed/Expected ratio. Due to the fact that the incidence of liver cancer was low in all studies, theses different measurements can be used to provide similar estimates of RR.

Methodological quality assessment

Of note, the included RCT was pooled analysis of other RCTs, therefore, it is inappropriate to assess the methodological quality. The methodological quality of cohort and case-control studies were assessed on the Newcastle-Ottawa Scale,[24] including eight items that were categorized three categories: selection (four items, one star each), comparability (one item, up to two stars), and exposure/outcome (three items, one star each). A "star" presents a "high" quality choice of each item.

Statistical analysis

The overall meta-analysis was first performed, followed by the subgroup analyses, based on study design, baseline risk of liver cancer, confounding adjustment, study location, and pharmacokinetic. Meanwhile, we conducted subgroup analyses based on studies which reported RR estimate for higher cumulative dosage of statin use, when appropriate data were available.

To take into account the heterogeneity and provide a more conservative estimate, the inverse variance method was used to estimate the pooled RR and corresponding 95%

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CIs, and data were pooled using a random effects model. Heterogeneity was assessed using the Chi-squared statistic (P) together with the Higgins I-squared statistic (I^2), a P value <0.10 was considered statistically significant for heterogeneity; and an I^2 value > 50 % was considered a measure of severe heterogeneity.[25]

Publication bias was assessed using the Begg's test and the Egger's test.[26] Influence analysis was performed to investigate the influence of a single study on the overall meta-analysis estimate, by omitting one study in each turn. Test for interaction was applied to identify the difference between pooled RR from subgroup analysis using the method described by Altman and Bland.[27] All statistical tests were two-sided and $P < 0.05$ was considered statistically significant, unless otherwise specified. Software Review Manager (RevMan5.2, Copenhagen) and STATA (Stata 11.2, Texas) were used for the statistical analysis.

Results

Study selection

Figure 1 illustrated the process of study selection for the meta-analysis. Of the 1424 potentially relevant references identified by electric and manual search, 142 were selected for full-text review after screening titles and abstracts. Finally, a total of 12 studies were included, with one IPD analysis,[19] five cohort studies,[28-32] and six case-control studies.[33-38] One case-control study was presented solely in abstract form.[33]

Of note, the cohort study conducted by Friedman *et al.* reported RR estimate separately for different gender (male and female),[29] we considered these two reports

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as separate studies. Therefore, a total of thirteen reports were included for the present meta-analysis.

Study characteristics

Table 1 summarized the characteristics of qualified studies in this meta-analysis. The 12 studies, involving 5,640,313 participants with 35,756 liver cancer cases, were published between 2005 and 2013. The "RCT" in the present study was pooled analysis of 22 clinical trials,[19] which investigated statins therapy in cardiovascular event prevention and reported the occurrence of liver cancer as adverse event. The observational studies were conducted with the local or national health databases, the statin exposure were identified by linkage to prescription databases, and the controls were matched mainly by age, sex and index date. Except one cohort adopted ICD-10 C22,[28] all other studies identified liver cancer cases according to the ICD-9 155. Of note, two cohorts were restricted to patients with HBV infection,[31] and HCV infection;[32] one case-control only included patients with diabetes mellitus;[34] two observational studies included patients aged at least 45 years.[30 35]

Table 2 summarized the data of the included studies. In the RCT[19] and one cohort study,[30] the RR with 95% CIs were calculated from the 2×2 tables defined by the incidence of liver cancer and the statin use status. The observational studies reported different measurements of RR estimates with adjustment by confounders. Several observational studies adopted the important risk factors of liver cancer for adjustments[31 32 34-36], such as HBV infection, HCV infection, cirrhosis, alcoholic liver disease, or non-alcoholic fatty liver disease (NAFLD).[39] Of note, only two studies

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adjusted for the cholesterol level,[30 38] and no study adjusted for the metabolic syndrome, which might also influence the risk of liver cancer.[39]

Methodological quality

For the cohort and case-control studies, the median score was 7 on the Newcastle-Ottawa Scale, with a range of 5 to 8 (**Supplementary Table 1**). These results indicated that the observational studies were in a reasonable good quality.

Overall meta-analysis

Figure 2 depicted the forest plot of RR estimate with 95% CIs from individual studies and overall meta-analysis. In the overall meta-analysis, pooled results showed a statistically significant decrease in the liver cancer risk among all statin users (RR 0.58, 95%CIs 0.51–0.67). Of note, a statistically significant heterogeneity was observed (I^2 = 65%, P = 0.0006). The P-values of Begg's test and Egger's test were 0.669 and 0.749, respectively, both suggesting there was no evidence of publication bias. In the influence analysis, the omission of any individual studies did not alter the direction and magnitude of the observed effect (**Supplementary Figure 1**).

Subgroup analyses and Test for interaction

We first performed preplanned subgroup analyses based on study design, baseline risk of liver cancer, confounding adjustment, and study location (**Table 3**).

The RCT showed there is no significant association between statin use and risk of liver cancer (RR 1.06, 0.66–1.71). But the observational studies indicated a significant decrease of liver cancer risk among all statin users (RR 0.57, 0.50–0.64; I^2 = 61%, P = 0.003) (**Figure 2**). Furthermore, we found a greater risk reduction in the subgroup

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analysis of cohort studies (RR 0.51, 0.44–0.58; I^2 = 18%, P = 0.30) than in the case-control studies (RR 0.63, 0.54–0.73; I^2 = 46%, P = 0.10) (**Supplementary Figure 2**).

Test for interaction showed significant results between subgroups of the RCT and observational studies ($P_{interaction}$ = 0.01, Z = 2.47), and between subgroups of the cohort and case-control studies ($P_{interaction}$ = 0.04, Z = -2.03). These results indicated that the difference of the study designs was the part reason that why there was severe heterogeneity in the overall analysis (**Table 3**).

In the subgroup analysis of the four studies with higher baseline risk of liver cancer,[30-32 35] defined as patients with older age, HBV or HCV infection, there was a trend toward more decrease of liver cancer risk (RR 0.52, 0.47-0.59; I^2 = 16%, P = 0.31) than in the other eight studies with general population[19 28 29 33 34 36-38] (RR 0.63, 0.52–0.75; I^2 = 59%, P = 0.01) (**Supplementary Figure 3**).

We defined the RCT or studies adjusted for at least 4 of 7 important confounders, such as HBV infection, HCV infection, cirrhosis, alcoholic liver disease, NAFLD, HBV treatment, or HCV treatment,[39] were adjusted adequately. Subgroup analysis of these six studies[19 31 32 34-36] found a trend toward less decrease of liver cancer risk (RR 0.64, 0.53-0.77; I^2 = 81% P = 0.0001) than the other six studies[28-30 33 37 38] (RR, 0.51, 0.43-0.60; I^2 = 3%, P = 0.40) (**Supplementary Figure 4**).

Subgroup analyses based on study location found a similar risk reduction of liver cancer in the Western countries (RR 0.61, 0.48–0.76; I^2 = 64%, P = 0.007) and in the Asian countries (RR 0.56, 0.48–0.64; I^2 = 51%, P = 0.09). (**Supplementary Figure 5**)

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Besides the overall RR estimates, some studies reported different RR estimate for different pharmacokinetic and dosage of statin use (**Supplementary Table 2)**. We conducted further subgroup analyses based on these available data.

According to the different pharmacokinetic, statins can be classified as lipophilic statins (Atorvastatin, Fluvastatin, Lovastatin, and Simvastatin) and hydrophilia statins (Pravastatin and Rosuvastatin).[21] Subgroup analysis of lipophilic statins [29 31 34-36] found a significant decrease of liver cancer risk (RR 0.57, 0.50–0.65; I^2 = 50%, P = 0.08). And there was a similar result among users of hydrophilia statins[31 35 36] (RR 0.59, 0.41–0.84; I^2 = 50%, P = 0.13) (**Supplementary Figure 6**).

Test for interaction showed non-significant results for subgroups with different baseline risk, confounding adjustment, study location, or pharmacokinetic ($P_{interaction}$ = 0.08, 0.08, 0.54 and 0.86, respectively) (**Table 3**). Therefore, there is no strong evidence to support a different preventive effect of statins on liver cancer in these subgroups.

Subgroup analysis of six studies with higher cumulative dose of statin use, defined as statin use more than 180 cumulative defined daily dose (cDDDs) or 0.5 years (cumulative duration), showed a trend toward more risk reduction of liver cancer (RR 0.53, 0.36-0.79), but with a high degree of heterogeneity (I^2 = 90%, P<0.00001) (**Supplementary Figure 7**).

Discussion

This present meta-analysis represents the most comprehensive review to date on the association between the statin use and the liver cancer risk, by including 12 studies

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(one IPD analysis of 22 RCTs, 5 cohort studies, and 6 case-control studies) and involving 5,640,313 participants with 35,756 liver cancer cases. Overall, we found that statin use was associated with an over 40% risk reduction in liver cancer compared with nonusers (RR 0.58, 95%CIs0.51–0.67). This result was in line with the previous three meta-analyses: Singh *et al.* included 10 studies and suggested statin users were less likely to develop HCC (OR 0.63, 95%CIs 0.52-0.76),[16] Pradelli *et al.* and Zhang *et al.* included 5 and 7 observational studies and found a summary RR of 0.58 (95%CIs 0.46–0.74) and 0.61 (95%CIs 0.49–0.76), respectively.[40 41]

The IPD analysis of 22 RCTs showed there is no significant association between statin use and risk of liver cancer. The significant risk reduction of liver cancer among all statin users was seen primarily in the observational studies, and this preventive effect was relatively convinced in the cohorts than in the case-controls. There were some reasons to explain the different findings between RCTs and observational studies.

First, the exposure period to statins might be shorter than the period to carcinogenesis and the latency to diagnosis in the cohorts and the case-controls. The observational studies defined statin use varying in dosage and duration, from patients who received ≥1 cDDD or >1 Rx of statins to more than 0.5 years (**Table1**). On the other hand, the median period of statin use was 5.1 years in the RCTs. Although there was a trend toward more risk reduction of liver cancer with higher cumulative dose of statin use, this defect might still result in overestimating the cancer-preventive effect of statins in the observational studies.

Second, clinical studies demonstrated that higher serum total cholesterol

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concentration was associated with decreased risk of liver cancer (**Supplementary Table 3**).[42-44] Meanwhile, there were inverse association between use of non-statin lipid-lowering drugs and risk of the liver cancer.[35 38] Meanwhile, because of the contraindication, statins might not prescribed to the patients with the chronic liver disease, which is known as a risk factor of liver cancer. Unfortunately, the observational studies included in this analysis seldom adopted these factors for adjustment. Actually, subgroup analysis of studies with adequate adjustment showed a trend toward less risk reduction, indicating the potential of overestimate this preventive effect by confounders.

Third, the RCTs included lower risk population (patients with cardiovascular disease rather than HBV /HCV infection), might not be powerful enough to investigate the liver cancer outcomes, which were much rarer than cardiovascular events. In addition, subgroup analysis of studies with higher baseline risk showed a trend toward more decrease of liver cancer risk.

These reasons suggested that the observed modulation of cancer incidence cannot be ascribable to a direct statin-mediated effect,[20] the exposure period, the indication (e.g. hyperlipidemia) and contraindication (e.g. chronic liver disease) of statins might overestimate its cancer-preventive effect.

We found similar results in Western countries and Asian countries, which were different from the meta-analysis conducted by Singh *et al.* which concluded that the inverse association of statins with HCC was stronger in the Asian population. Considering four more studies we included, this difference might be caused by the

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insufficient data in their meta-analysis. Based on the pharmacokinetics, it is plausible that lipophilic and hydrophilic statins will differ in their liver cancer prevention qualities.[21 22] However, subgroup analysis of lipophilic and hydrophilic statins showed similar results.

Besides the limitations described previously, there were some other limitations should be noted. First, a significant heterogeneity was observed in the present meta-analysis, which might results from the difference in study design. Results of subgroup analyses would also be limited by this heterogeneity. Second, the adherence to statin therapy is known to be associated with healthy lifestyle, which might affect the cancer outcome.[45] Such information is hard to be captured in databases or medical record in the observational studies.[46] Third, five observational studies were conducted using the Taiwanese National Health Insurance Research Database (NHIRD),[31 32 35-37] although they were not in the same period, these studies might contain overlapping groups of patients. These limitations mentioned above might lead to confounding of overall results from the present study, and should be considered in future studies aiming at confirming the protective effects of statins on human cancer risk.

The strengths of our meta-analysis were as follows: First, we performed a much more comprehensive search and more subgroup analyses, compared with the previous meta-analyses; Second, the methodological quality of the included studies were reasonable good; Third, publication bias, which due to the tendency of not publishing small studies with null results, were not found in our meta-analysis.

Of note, preclinical studies have indicated that statins possess synergism with other

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therapeutic agents *in vitro* and *in vivo* for liver cancer.[47 48] Some clinical studies have also demonstrated that statins would prolong survival in patients with advanced liver cancer (**Supplementary Table 4**),[49-52] and associated with risk reduction of recurrence after curative surgery in patients of HBV related HCC.[53] Therefore, considerable interest exists in adjunctive therapy with statins for liver cancer. In fact, there were some RCTs ongoing to determine the effectiveness of pravastatin, when used in combination with sorafenib, in the treatment of liver cancer (**Supplementary Table 5**).

Currently, physicians are less likely to prescribe statins for patients with chronic liver disease, based on the concerns about the statin-induced liver injury.[31] However, there were number of studies have demonstrated the safe use, even salutary effects.[54-56] Meanwhile, the risk of serious statin-related liver injury appears to be no greater than the background incidence of this rare event.[57] Therefore, considering their benefits for cardiovascular event prevention and the potential effect in liver cancer prevention and treatment, statins should not be denied to these patients.

In conclusion, our results suggest that statin use is associated with a significant risk reduction of liver cancer, when taken daily for cardiovascular event prevention. However, this preventive effect might be overestimated due to the exposure period, indication and contraindication of statins, and other confounders. Statins might be considered as an adjuvant in the treatment of liver cancer.

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CONTRIBUTORSHIP STATEMENT

XB Cui had the original idea, Meng Shi and XB Cui worked together to develop an appropriate theoretical framework and design. XB Cui developed the search, Meng Shi and XB Cui were involved in the selection process. Meng Shi and XB Cui extracted relevant data, XB Cui and Biao Nie performed the statistical analysis and all authors were involved in the data interpretation. Meng Shi and Biao Nie wrote the manuscript draft and revised the draft based on input from the other authors. All authors revised it critically for content and approved the final version.

COMPETING INTERESTS

There are no competing interests

FUNDING

None.

DATA SHARING

No additional data available.

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Figure legends:

Figure 1. Flow chart of study selection in the present meta-analysis.

Figure 2. Overall meta-analysis of the statin use and the liver cancer risk.

Supplementary Figure 1. Influence analysis.

Supplementary Figure 2. Subgroup analyses based on study design.

Supplementary Figure 3. Subgroup analyses based on baseline risk of liver cancer.

Supplementary Figure 4. Subgroup analyses based on confounder adjustment.

Supplementary Figure 5. Subgroup analyses based on study location.

Supplementary Figure 6. Subgroup analyses based on pharmacokinetic of statins.

Supplementary Figure 7. Subgroup analysis of higher cumulative dose of statin use.

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Table 1. Study characteristics

Studies	Study design	Patient population	Study period	Cases defined	Follow-up	Statins type	Dosage/Duration of Statin use
Emberson, 2012, UK [19]	RCT	IPD analysis of 22 RCTs	-	ICD-9 155	5.1 years (Me)	A, F, L, P, R, S	5.1 years (Me)
Friis, 2005, North Jutland [28]	Cohort	General population (CPR)	1989-2002	ICD-10 C22	3.3 years (M)	Unspecified	≥2 Rx
Friedman, 2008, USA [29]	Cohort	General population (KPMCP)	1994-2003	ICD-9-CM 155	> 2 years	A, L, S (97.6%)	≥1 Rx
Marelli, 2011, USA [30]	Cohort	General older population (men ≥ 45 and women ≥ 55 years; GE Centricity)	1990-2009	ICD-9 155	4.6 years (M)	Unspecified	≥1 cDDD
Tsan, 2012, Taiwan [31]	Cohort	Patients with HBV infection (NHIRD)	1997-2008	ICD-9 155	9.9 years (M)	A, F, L, P, R, and S	≥28 cDDDs
Tsan, 2013, Taiwan [32]	Cohort	Patients with HCV infection (NHIRD)	1999-2010	ICD-9 155	10.7 years (M)	A, F, L, P, R, and S	≥28 cDDDs
Khurana, 2005, USA [33]	Case control	General population (VISN)	1997-2002	ICD-9 155	NR	Unspecified	≥1 Rx
El-Serag, 2009, USA [34]	Case control	Diabetes patients (VA)	1997-2002	ICD-9-CM 155	2.4 years (M)	A, C, F, L, P, and S	1.6 years (M)
Chiu, 2011, Taiwan [35]	Case control	Older patients(≥ 50 years; NHIRD)	2005–2008	ICD-9-CM 155	NR	A, F, L, P, R, and S	≥ 1 cDDD
Lai, 2013, Taiwan [36]	Case control	General population (NHIRD)	2000-2009	ICD-9-CM 155	1.4 years (M)	A, F, L, P, R, and S	≥1 Rx
Leung, 2013, Taiwan [37]	Case control	General population (NHIRD)	2000-2008	ICD-9-CM 155	4.1 years (M)	Unspecified	> 0.5 years
Chaiteerakij, 2013, USA [38]	Case control	Hyperlipidemia patients (Mayo Clinic)	2000-2010	ICD-9-CM 155	>1 years	Unspecified	≥1 Rx

Patients population: IPD = Individual patient data, RCT = randomized controlled trials, CRP = the Central Population Register of Danish citizens, KPMCP = the Kaiser Permanente Medical Care Program in northern California, GE Centricity = the General Electric Centricity database, NHIRD = the Taiwanese National Health Insurance research database, VISN = Veterans Integrated Service Networks 16 Veteran Affairs database, VA = Veterans Affairs national databases, Mayo Clinic = Mayo Clinic (Rochester, MN), HBV = hepatitis B virus; Cases defined: ICD-9 or -10 = International Classification of Diseases, Ninth Revision or Tenth Revision, CM = Clinical Modification; Duration of follow-up: When the follow-up periods of statin user and nonuser were different, only the shorter one was showed, and all periods were transformed to years; Statin type: A = Atorvastatin, C = Cerivastatin, F = Fluvastatin, L = Lovastatin, P = Pravastatin, R = Rosuvastatin, S = Simvastatin, Non-statin = Non-statin cholesterol-lowering drug(s) only; Duration of statin use: M = Mean, Me = Median, ≥1 cDDD = more than 1 cumulative defined daily dose before the diagnosis of liver cancer, Rx = prescriptions.

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Table 2. Study data

Studies	Intervention/ Cases		Control		Measurements of effect estimates	Crude RR with 95% CIs	Adjusted RR with 95% CIs	Confounders for adjustment
	No. of event/ No. of exposure	No. of total	No. of event/ No. of exposure	No. of total				
Emberson, 2012, UK [19]	35	67258	33	67279	RR	1.06 (0.66, 1.71)*	1.06 (0.66, 1.71)*	Randomization
Friis, 2005, North Jutland [28]	1	12251	166	334754	OR	NA	1.16 (0.46-2.90)	1,2, 16, 21, 23
Friedman(Male), 2008, USA [29]	32	192598	NA	1904876	HR	NA	0.49 (0.34-0.70)	16
Friedman(Female), 2008, USA [29]	10	169261	NA	1976332	HR	NA	0.40 (0.21-0.75)	
Marelli, 2011, USA [30]	13	45857	24	45857	RR	0.31 (0.14-0.68)*	0.31 (0.14-0.68)*	1-5, 14, 16-18, 26, 27
Tsan, 2012, Taiwan [31]	58	2785	963	30628	HR	0.66 (0.51- 0.86)	0.47 (0.36-0.61)	1, 2, 7, 8, 11, 12
Tsan, 2013, Taiwan [32]	1378	35023	26505	225841	HR	0.42 (0.39-0.46)	0.53 (0.49–0.58)	1, 2, 7, 8, 11, 13
Khurana, 2005, USA [33]	NA	NA	NA	NA	OR	NA	0.52 (0.41- 0.67)	1, 11, 13
El-Serag, 2009, USA [34]	447	1303	2766	5212	OR	0.46 (0.40-0.52)	0.74 (0.64-0.87)	1-3, 6, 8, 9, 11-13, 21, 24, 28
Chiu, 2011, Taiwan [35]	117	1166	195	1166	OR	0.53 (0.41-0.69)	0.62 (0.45-0.83)	1, 2, 8, 9, 11, 12, 20, 29
Lai, 2013, Taiwan [36]	255	3480	1635	13920	OR	0.61 (0.52–0.72)	0.71 (0.56–0.89)	1, 2, 8-13, 22, 24, 25
Leung, 2013, Taiwan [37]	26	424	6851	33781	HR	0.45 (0.30-0.67)	0.44 (0.28, 0.72)	1, 2, 11, 15, 20, 21, 23
Chaiteerakij, 2013, USA [38]	72	165	165	256	OR	NA	0.6 (0.4-0.9)	1-3, 8, 11, 17, 22, 28, 30

The RR with an asterisk mark (*) was calculated based on the raw data. The others, crude or adjusted, were extracted from the original paper; Confounders for adjustment: 1 = age, 2 = sex, 3 = race, 4 = BMI, 5 = smoking status, 6 = ethanol intake, 7 = socioeconomic status, 8 = cirrhosis, 9 = alcoholic liver disease, 10 = non-alcoholic fatty liver disease, 11 = diabetes mellitus, 12 = HBV infection, 13 = HCV infection, 14 = concomitant diagnoses (unspecified), 15 = Charlson score, 16 = calendar year, 17 = cholesterol (totalcholesterol, VLDL, LDL, or triglycerides), 18 = prostate-specific antigen, 19 = resection extent, 20 = other lipid-lowering agents, 21 = cardiovascular medications (aspirin, nonsteroidal anti-inflammatory medications, or angiotensin-converting enzymes inhibitors), 22 = metformin or thiazolidinedione, 23 = hormone-replacement therapy, 24 = HCV treatment, 25 = HBV treatment, 26 = medications taken (unspecified), 27 = the number of office visits, 28 = propensity to use statins, 29 = hospital stay, 30 = biliary tract diseases

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Table 3. Subgroup analyses of included studies

Subgroup		No. of studies (reports)	Summary RR (95% CIs)	Heterogeneity, I^2	Heterogeneity, *P* value	$P_{interaction}$
Study design	RCT	1	1.06 (0.66-1.71)	-	-	***P = 0.01***
	Observational studies	11(12)	0.57(0.50-0.64)	61%	***P* = 0.003**	
Observational studies	Cohort studies	5 (6)	0.51 (0.44–0.58)	18%	*P* = 0.30	***P* = 0.04**
	Case-control studies	6	0.63 (0.54–0.73)	46%	***P* = 0.10**	
Baseline risk of liver cancer	Higher baseline risk	4	0.52 (0.47-0.59)	16%	*P* = 0.31	***P*** = 0.08
	General population	8 (9)	0.63 (0.52–0.75)	59%	***P* = 0.01**	
Confounding adjustment	Adequate adjustment	6	0.64(0.53-0.77)	81%	***P* = 0.0001**	***P*** = 0.08
	Inadequate adjustment	6 (7)	0.51 (0.43-0.60)	3%	*P* = 0.40	
Study location	Western studies	8 (9)	0.61 (0.48-0.76)	64%	***P* = 0.007**	*P* = 0.54
	Asian studies	6	0.56 (0.48- 0.64)	51%	***P* = 0.09**	
Pharmacokinetic	Hipophilic statins	5 (6)	0.57 (0.50-0.65)	50%	***P* = 0.08**	***P*** = 0.86
	Hydrophilia statins	3	0.59(0.41–0.84)	50%	***P* = 0.13**	
Higher cumulative dosage of statin		6	0.53 (0.36-0.79)	90%	***P*<0.0001**	-

RR = relative risk; higher baseline risk of liver cancer: patients with older age, HBV or HCV infection. Adequate adjustment: RCT or studies which adjusted for at least 4 of 7 important confounders, such as HBV infection, HCV infection, cirrhosis, alcoholic liver disease, NAFLD, HBV treatment, or HCV treatment; Lipophilic statins: Atorvastatin, Fluvastatin, Lovastatin, or Simvastatin; Hydrophilia statins: Pravastatin or Rosuvastatin; Higher cumulative dosage of statin use: > 180cumulative defined daily dose or Duration of statin use > 0.5 years before the diagnosis of liver cancer.

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Statin use and risk of liver cancer: an update meta-analysis

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Running title: Meta-analysis: statin and liver cancer

Key words: *Statin; Liver cancer; Cancer Prevention; Meta-analysis.*

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Abstract

Objective: Statins are commonly prescribed cholesterol-lowering drugs. Preclinical studies suggest that statins may possess cancer preventive properties. The primary objective of this meta-analysis was to determine the association between the statin use and the risk of liver cancer.

Design: Meta-analysis.

Setting: International.

Participants: A comprehensive literature search of PubMed, BIOSIS Previews, Web of Science, EMBASE, EBSCO and Cochrane Library was conducted through March 2014. The effect estimate was reported as pooled relative risk (RR) with 95% confidence intervals (CIs), using the random-effects model.

Results: A total of 12 studies (one individual patient data analysis of 22 randomized controlled trials, 5 cohorts, and 6 case-controls) were qualified for this meta-analysis, involving 5,640,313 participants including 35,756 liver cancer cases. Our results indicated a significant risk reduction of liver cancer among all statin users (RR 0.58, 95%CIs 0.51–0.67). The difference of the study designs can partly explained the significant heterogeneity found in the overall analysis (I^2 = 65%, P = 0.0006). No evidence of publication bias was observed in this meta-analysis. Similar risk reductions were found in the subgroups analysis of Western and Asian countries, lipophilic and hydrophilia statins. There was a trend toward more risk reductions in subgroups with higher baseline risk, inadequate adjustment, and higher cumulative dosage of statin use.

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Conclusions: This meta-analysis suggests that the statin is associated with a significant risk reduction of liver cancer, when taken daily for cardiovascular event prevention. However, this preventive effect might be overestimated due to the exposure period, tthe indication and contraindication of statins, and other confounders. Statins might be considered as an adjuvant in the treatment of liver cancer.

Key words: *Statin; Liver cancer; Cancer Prevention; Meta-analysis.*

Strengths and limitations of this study

Statins are commonly prescribed as cholesterol-lowering drugs. In this comprehensive meta-analysis, we demonstrate that the statin use is associated with a significant risk reduction of liver cancer.

The difference of the study designs is the part reason that explained the significant heterogeneity found in the overall analysis.

However, this preventive effect might be overestimated due to the exposure period, tthe indication and contraindication of statins, and other confounders.

Statins might be considered as an adjuvant in the treatment of liver cancer.

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Introduction

Statins are inhibitors of 3-hydroxy-3-methylglutaryl coenzyme-A (HMG-CoA) reductase and they are widely used to reduce the plasma cholesterol level and the risk of cardiovascular events.[1] Although there is a concern over their possible carcinogenicity raised in rodent studies,[2] preclinical studies indicate that statins have anticancer properties *in vitro* and *in vivo*, through inhibiting angiogenesis, inducing apoptosis, and suppressing tumor growth and metastasis.[3-5]

However, higher concentrations of statins are typically required to induce these effects, raising questions concerning the therapeutic relevance of statins on cancer.[6] To date, clinical studies regarding the cancer incidence associated with statin administration have highlighted conflicting results. Moreover, a large number of meta-analyses have concluded that there was no association between statin use and risk of overall cancer,[7-10] or cancer of breast[11], stomach,[12] or pancreas.[13] There is only a modest protective effect of statins in prostate cancer[14] and colorectal cancer.[15]

In contrary, recent studies reported encouraging results for risk reduction of liver cancer among all statin users. Previous meta-analysis, conducted by Singh *et al*. by including 10 studies, found that statin users were less likely to develop hepatocellular carcinoma (HCC) than statin non-users.[16] However, Singh *et al*. included the ALERT, LIPS, and MEGA trials twice, by including three individual patient data (IPD) analysis of randomized controlled trials (RCTs).[17-19] Meanwhile, some factors of stratification were not considered in their analyses, such as dose and timing of exposure to statins, and the selection of controls and confounders, which might limit

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the evaluation of cancer risk.[20] Furthermore, the lipophilic statins are accompanied by an extensive first-pass effect at the hepatic level.[21] It is plausible that lipophilic statins may have a better liver cancer preventive qualities than the hydrophilic ones.[22]

Therefore, we performed this updated meta-analysis to assess the association between the statin use and the risk of liver cancer, involving the recently published studies and conducting more subgroup analyses based on the factors mentioned above. Our results demonstrated that statin use was associated with an over 40% risk reduction in liver cancer, which may have a significant translational potential in the clinical practice. However, there were some confounders might overestimate this preventive effect of statins.

MATERIALS AND METHODS

Literature Search strategy

This meta-analysis was conducted following the PRISMA guidelines.[23]

The systematic computerized search for eligible studies were performed on the database of PubMed, BIOSIS Previews, Web of Science, EMBASE, EBSCO, and Cochrane Library, covering all studies published from their inception to March 5, 2014. The following terms were searched with both the subjects (MeSH terms) and text-word search strategies: "(Statin OR HMG-CoA reductase inhibitors OR Atorvastatin OR Cerivastatin OR Fluvastatin OR Lovastatin OR Pravastatin OR Rosuvastatin OR Simvastatin) AND (Hepatocellular OR Hepatic OR Intrahepatic OR Interlobular OR Liver) AND (Carcinoma OR Sarcomas OR Angiosarcoma OR Cancer OR Neoplasm). Additionally, the relevant reviews and retrieved articles were searched

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manually for more eligible studies.

In study searching, only the original researches, published in form of peer review article or meeting abstract, were included. No language restrictions were imposed. However, the studies we included were all published in English.

Study selection

The inclusion criteria were: (1) randomized controlled trial (RCTs), cohort studies or case-control studies; (2) original studies that assessed the effect of statin use on the risk of liver cancer, compared with placebo or no treatment; (3) liver cancer cases were identified according to the International Classification of Diseases codes (ICD); and (4) studies with estimate of relative risk (risk ratio, RR) of liver cancer, or with data sufficient to calculate it.

The exclusion criteria were: (1) study design not meeting the inclusion criteria; (2) studies without estimate of RR, or without sufficient data to calculate it; or (3) studies with duplicated or overlap reports.

Data extraction

Two independent investigators (M. Shi and X.B. Cui) extracted data from the eligible studies using a predefined data collection form. The differences of data extraction were resolved by consensus referring back to the original article. The extracted information included: (1) Studies: first author, year of publication, study design, location, patient populations, period, and follow-up; (2) Statins: type, dosage or duration of statin use; (3) liver cancer: case identification, number of liver cancer, crude RR with 95% confidence intervals (CIs), adjusted RR reflecting the greatest

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degree of control for confounders, and confounders for adjustment (including variables for matching). When the RR were not available, the RR with 95% CIs were calculated from the raw data in original studies.

We extracted different measurements of effect estimates from original studies, such as Relative Risk (RR), Odds Ratio (OR), Hazard Ratio (HR), and Observed/Expected ratio. Due to the fact that the incidence of liver cancer was low in all studies, theses different measurements can be used to provide similar estimates of RR.

Methodological quality assessment

Of note, the included RCT was pooled analysis of other RCTs, therefore, it is inappropriate to assess the methodological quality. The methodological quality of cohort and case-control studies were assessed on the Newcastle-Ottawa Scale,[24] including eight items that were categorized three categories: selection (four items, one star each), comparability (one item, up to two stars), and exposure/outcome (three items, one star each). A "star" presents a "high" quality choice of each item.

Statistical analysis

The overall meta-analysis was first performed, followed by the subgroup analyses, based on study design, baseline risk of liver cancer, confounding adjustment, study location, and pharmacokinetic. Meanwhile, we conducted subgroup analyses based on studies which reported RR estimate for higher cumulative dosage of statin use, when appropriate data were available.

To take into account the heterogeneity and provide a more conservative estimate, the inverse variance method was used to estimate the pooled RR and corresponding 95%

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CIs, and data were pooled using a random effects model. Heterogeneity was assessed using the Chi-squared statistic (P) together with the Higgins I-squared statistic (I^2), a P value <0.10 was considered statistically significant for heterogeneity; and an I^2 value > 50 % was considered a measure of severe heterogeneity.[25]

Publication bias was assessed using the Begg's test and the Egger's test.[26] Influence analysis was performed to investigate the influence of a single study on the overall meta-analysis estimate, by omitting one study in each turn. Test for interaction was applied to identify the difference between pooled RR from subgroup analysis using the method described by Altman and Bland.[27] All statistical tests were two-sided and $P < 0.05$ was considered statistically significant, unless otherwise specified. Software Review Manager (RevMan5.2, Copenhagen) and STATA (Stata 11.2, Texas) were used for the statistical analysis.

Results

Study selection

Figure 1 illustrated the process of study selection for the meta-analysis. Of the 1424 potentially relevant references identified by electric and manual search, 142 were selected for full-text review after screening titles and abstracts. Finally, a total of 12 studies were included, with one IPD analysis,[19] five cohort studies,[28-32] and six case-control studies.[33-38] One case-control study was presented solely in abstract form.[33]

Of note, the cohort study conducted by Friedman *et al.* reported RR estimate separately for different gender (male and female),[29] we considered these two reports

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as separate studies. Therefore, a total of thirteen reports were included for the present meta-analysis.

Study characteristics

Table 1 summarized the characteristics of qualified studies in this meta-analysis. The 12 studies, involving 5,640,313 participants with 35,756 liver cancer cases, were published between 2005 and 2013. The "RCT" in the present study was pooled analysis of 22 clinical trials,[19] which investigated statins therapy in cardiovascular event prevention and reported the occurrence of liver cancer as adverse event. The observational studies were conducted with the local or national health databases, the statin exposure were identified by linkage to prescription databases, and the controls were matched mainly by age, sex and index date. Except one cohort adopted ICD-10 C22,[28] all other studies identified liver cancer cases according to the ICD-9 155. Of note, two cohorts were restricted to patients with HBV infection,[31] and HCV infection;[32] one case-control only included patients with diabetes mellitus;[34] two observational studies included patients aged at least 45 years.[30 35]

Table 2 summarized the data of the included studies. In the RCT[19] and one cohort study,[30] the RR with 95% CIs were calculated from the 2×2 tables defined by the incidence of liver cancer and the statin use status. The observational studies reported different measurements of RR estimates with adjustment by confounders. Several observational studies adopted the important risk factors of liver cancer for adjustments[31 32 34-36], such as HBV infection, HCV infection, cirrhosis, alcoholic liver disease, or non-alcoholic fatty liver disease (NAFLD).[39] Of note, only two studies

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adjusted for the cholesterol level,[30 38] and no study adjusted for the metabolic syndrome, which might also influence the risk of liver cancer.[39]

Methodological quality

For the cohort and case-control studies, the median score was 7 on the Newcastle-Ottawa Scale, with a range of 5 to 8 (**Supplementary Table 1**). These results indicated that the observational studies were in a reasonable good quality.

Overall meta-analysis

Figure 2 depicted the forest plot of RR estimate with 95% CIs from individual studies and overall meta-analysis. In the overall meta-analysis, pooled results showed a statistically significant decrease in the liver cancer risk among all statin users (RR 0.58, 95%CIs 0.51–0.67). Of note, a statistically significant heterogeneity was observed (I^2 = 65%, P = 0.0006). The P-values of Begg's test and Egger's test were 0.669 and 0.749, respectively, both suggesting there was no evidence of publication bias. In the influence analysis, the omission of any individual studies did not alter the direction and magnitude of the observed effect (**Supplementary Figure 1**).

Subgroup analyses and Test for interaction

We first performed preplanned subgroup analyses based on study design, baseline risk of liver cancer, confounding adjustment, and study location (**Table 3**).

The RCT showed there is no significant association between statin use and risk of liver cancer (RR 1.06, 0.66–1.71). But the observational studies indicated a significant decrease of liver cancer risk among all statin users (RR 0.57, 0.50–0.64; I^2 = 61%, P = 0.003) (**Figure 2**). Furthermore, we found a greater risk reduction in the subgroup

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analysis of cohort studies (RR 0.51, 0.44–0.58; I^2 = 18%, P = 0.30) than in the case-control studies (RR 0.63, 0.54–0.73; I^2 = 46%, P = 0.10) (**Supplementary Figure 2**).

Test for interaction showed significant results between subgroups of the RCT and observational studies ($P_{interaction}$ = 0.01, Z = 2.47), and between subgroups of the cohort and case-control studies ($P_{interaction}$ = 0.04, Z = -2.03). These results indicated that the difference of the study designs was the part reason that why there was severe heterogeneity in the overall analysis (**Table 3**).

In the subgroup analysis of the four studies with higher baseline risk of liver cancer,[30-32 35] defined as patients with older age, HBV or HCV infection, there was a trend toward more decrease of liver cancer risk (RR 0.52, 0.47-0.59; I^2 = 16%, P = 0.31) than in the other eight studies with general population[19 28 29 33 34 36-38] (RR 0.63, 0.52–0.75; I^2 = 59%, P = 0.01) (**Supplementary Figure 3**).

We defined the RCT or studies adjusted for at least 4 of 7 important confounders, such as HBV infection, HCV infection, cirrhosis, alcoholic liver disease, NAFLD, HBV treatment, or HCV treatment,[39] were adjusted adequately. Subgroup analysis of these six studies[19 31 32 34-36] found a trend toward less decrease of liver cancer risk (RR 0.64, 0.53-0.77; I^2 = 81% P = 0.0001) than the other six studies[28-30 33 37 38] (RR, 0.51, 0.43-0.60; I^2 = 3%, P = 0.40) (**Supplementary Figure 4**).

Subgroup analyses based on study location found a similar risk reduction of liver cancer in the Western countries (RR 0.61, 0.48–0.76; I^2 = 64%, P = 0.007) and in the Asian countries (RR 0.56, 0.48–0.64; I^2 = 51%, P = 0.09). (**Supplementary Figure 5**)

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Besides the overall RR estimates, some studies reported different RR estimate for different pharmacokinetic and dosage of statin use (**Supplementary Table 2**). We conducted further subgroup analyses based on these available data.

According to the different pharmacokinetic, statins can be classified as lipophilic statins (Atorvastatin, Fluvastatin, Lovastatin, and Simvastatin) and hydrophilia statins (Pravastatin and Rosuvastatin).[21] Subgroup analysis of lipophilic statins [29 31 34-36] found a significant decrease of liver cancer risk (RR 0.57, 0.50–0.65; I^2 = 50%, P = 0.08). And there was a similar result among users of hydrophilia statins[31 35 36] (RR 0.59, 0.41–0.84; I^2 = 50%, P = 0.13) (**Supplementary Figure 6**).

Test for interaction showed non-significant results for subgroups with different baseline risk, confounding adjustment, study location, or pharmacokinetic ($P_{interaction}$ = 0.08, 0.08, 0.54 and 0.86, respectively) (**Table 3**). Therefore, there is no strong evidence to support a different preventive effect of statins on liver cancer in these subgroups.

Subgroup analysis of six studies with higher cumulative dose of statin use, defined as statin use more than 180 cumulative defined daily dose (cDDDs) or 0.5 years (cumulative duration), showed a trend toward more risk reduction of liver cancer (RR 0.53, 0.36-0.79), but with a high degree of heterogeneity (I^2 = 90%, P<0.00001) (**Supplementary Figure 7**).

Discussion

This present meta-analysis represents the most comprehensive review to date on the association between the statin use and the liver cancer risk, by including 12 studies

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(one IPD analysis of 22 RCTs, 5 cohort studies, and 6 case-control studies) and involving 5,640,313 participants with 35,756 liver cancer cases. Overall, we found that statin use was associated with an over 40% risk reduction in liver cancer compared with nonusers (RR 0.58, 95%CIs0.51–0.67). This result was in line with the previous three meta-analyses: Singh *et al.* included 10 studies and suggested statin users were less likely to develop HCC (OR 0.63, 95%CIs 0.52-0.76),[16] Pradelli *et al.* and Zhang *et al.* included 5 and 7 observational studies and found a summary RR of 0.58 (95%CIs 0.46–0.74) and 0.61 (95%CIs 0.49–0.76), respectively.[40 41]

The IPD analysis of 22 RCTs showed there is no significant association between statin use and risk of liver cancer. The significant risk reduction of liver cancer among all statin users was seen primarily in the observational studies, and this preventive effect was relatively convinced in the cohorts than in the case-controls. There were some reasons to explain the different findings between RCTs and observational studies.

First, the exposure period to statins might be shorter than the period to carcinogenesis and the latency to diagnosis in the cohorts and the case-controls. The observational studies defined statin use varying in dosage and duration, from patients who received ≥1 cDDD or >1 Rx of statins to more than 0.5 years (**Table1**). On the other hand, the median period of statin use was 5.1 years in the RCTs. Although there was a trend toward more risk reduction of liver cancer with higher cumulative dose of statin use, this defect might still result in overestimating the cancer-preventive effect of statins in the observational studies.

Second, clinical studies demonstrated that higher serum total cholesterol

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concentration was associated with decreased risk of liver cancer (**Supplementary Table 3**).[42-44] Meanwhile, there were inverse association between use of non-statin lipid-lowering drugs and risk of the liver cancer.[35 38] Meanwhile, because of the contraindication, statins might not prescribed to the patients with the chronic liver disease, which is known as a risk factor of liver cancer. Unfortunately, the observational studies included in this analysis seldom adopted these factors for adjustment. Actually, subgroup analysis of studies with adequate adjustment showed a trend toward less risk reduction, indicating the potential of overestimate this preventive effect by confounders.

Third, the RCTs included lower risk population (patients with cardiovascular disease rather than HBV /HCV infection), might not be powerful enough to investigate the liver cancer outcomes, which were much rarer than cardiovascular events. In addition, subgroup analysis of studies with higher baseline risk showed a trend toward more decrease of liver cancer risk.

These reasons suggested that the observed modulation of cancer incidence cannot be ascribable to a direct statin-mediated effect,[20] the exposure period, the indication (e.g. hyperlipidemia) and contraindication (e.g. chronic liver disease) of statins might overestimate its cancer-preventive effect.

We found similar results in Western countries and Asian countries, which were different from the meta-analysis conducted by Singh *et al.* which concluded that the inverse association of statins with HCC was stronger in the Asian population. Considering four more studies we included, this difference might be caused by the

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insufficient data in their meta-analysis. Based on the pharmacokinetics, it is plausible that lipophilic and hydrophilic statins will differ in their liver cancer prevention qualities.[21 22] However, subgroup analysis of lipophilic and hydrophilic statins showed similar results.

Besides the limitations described previously, there were some other limitations should be noted. First, a significant heterogeneity was observed in the present meta-analysis, which might results from the difference in study design. Results of subgroup analyses would also be limited by this heterogeneity. Second, the adherence to statin therapy is known to be associated with healthy lifestyle, which might affect the cancer outcome.[45] Such information is hard to be captured in databases or medical record in the observational studies.[46] Third, five observational studies were conducted using the Taiwanese National Health Insurance Research Database (NHIRD),[31 32 35-37] although they were not in the same period, these studies might contain overlapping groups of patients. These limitations mentioned above might lead to confounding of overall results from the present study, and should be considered in future studies aiming at confirming the protective effects of statins on human cancer risk.

The strengths of our meta-analysis were as follows: First, we performed a much more comprehensive search and more subgroup analyses, compared with the previous meta-analyses; Second, the methodological quality of the included studies were reasonable good; Third, publication bias, which due to the tendency of not publishing small studies with null results, were not found in our meta-analysis.

Of note, preclinical studies have indicated that statins possess synergism with other

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therapeutic agents *in vitro* and *in vivo* for liver cancer.[47 48] Some clinical studies have also demonstrated that statins would prolong survival in patients with advanced liver cancer (**Supplementary Table 4**),[49-52] and associated with risk reduction of recurrence after curative surgery in patients of HBV related HCC.[53] Therefore, considerable interest exists in adjunctive therapy with statins for liver cancer. In fact, there were some RCTs ongoing to determine the effectiveness of pravastatin, when used in combination with sorafenib, in the treatment of liver cancer (**Supplementary Table 5**).

Currently, physicians are less likely to prescribe statins for patients with chronic liver disease, based on the concerns about the statin-induced liver injury.[31] However, there were number of studies have demonstrated the safe use, even salutary effects.[54-56] Meanwhile, the risk of serious statin-related liver injury appears to be no greater than the background incidence of this rare event.[57] Therefore, considering their benefits for cardiovascular event prevention and the potential effect in liver cancer prevention and treatment, statins should not be denied to these patients.

In conclusion, our results suggest that statin use is associated with a significant risk reduction of liver cancer, when taken daily for cardiovascular event prevention. However, this preventive effect might be overestimated due to the exposure period, indication and contraindication of statins, and other confounders. Statins might be considered as an adjuvant in the treatment of liver cancer.

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CONTRIBUTORSHIP STATEMENT

XB Cui had the original idea, Meng Shi and XB Cui worked together to develop an appropriate theoretical framework and design. XB Cui developed the search, Meng Shi and XB Cui were involved in the selection process. Meng Shi and XB Cui extracted relevant data, XB Cui and Biao Nie performed the statistical analysis and all authors were involved in the data interpretation. Meng Shi and Biao Nie wrote the manuscript draft and revised the draft based on input from the other authors. All authors revised it critically for content and approved the final version.

COMPETING INTERESTS

There are no competing interests

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No additional data available.

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Figure legends:

Figure 1. Flow chart of study selection in the present meta-analysis.

Figure 2. Overall meta-analysis of the statin use and the liver cancer risk.

Supplementary Figure 1. Influence analysis.

Supplementary Figure 2. Subgroup analyses based on study design.

Supplementary Figure 3. Subgroup analyses based on baseline risk of liver cancer.

Supplementary Figure 4. Subgroup analyses based on confounder adjustment.

Supplementary Figure 5. Subgroup analyses based on study location.

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Supplementary Figure 6. Subgroup analyses based on pharmacokinetic of statins.

Supplementary Figure 7. Subgroup analysis of higher cumulative dose of statin use.

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Table 1. Study characteristics

Studies	Study design	Patient population	Study period	Cases defined	Follow-up	Statins type	Dosage/Duration of Statin use
Emberson, 2012, UK [19]	RCT	IPD analysis of 22 RCTs	-	ICD-9 155	5.1 years (Me)	A, F, L, P, R, S	5.1 years (Me)
Friis, 2005, North Jutland [28]	Cohort	General population (CPR)	1989-2002	ICD-10 C22	3.3 years (M)	Unspecified	≥2 Rx
Friedman, 2008, USA [29]	Cohort	General population (KPMCP)	1994-2003	ICD-9-CM 155	> 2 years	A, L, S (97.6%)	≥1 Rx
Marelli, 2011, USA [30]	Cohort	General older population (men ≥ 45 and women ≥ 55 years; GE Centricity)	1990-2009	ICD-9 155	4.6 years (M)	Unspecified	≥1 cDDD
Tsan, 2012, Taiwan [31]	Cohort	Patients with HBV infection (NHIRD)	1997-2008	ICD-9 155	9.9 years (M)	A, F, L, P, R, and S	≥28 cDDDs
Tsan, 2013, Taiwan [32]	Cohort	Patients with HCV infection (NHIRD)	1999-2010	ICD-9 155	10.7 years (M)	A, F, L, P, R, and S	≥28 cDDDs
Khurana, 2005, USA [33]	Case control	General population (VISN)	1997-2002	ICD-9 155	NR	Unspecified	≥1 Rx
El-Serag, 2009, USA [34]	Case control	Diabetes patients (VA)	1997-2002	ICD-9-CM 155	2.4 years (M)	A, C, F, L, P, and S	1.6 years (M)
Chiu, 2011, Taiwan [35]	Case control	Older patients(≥ 50 years; NHIRD)	2005–2008	ICD-9-CM 155	NR	A, F, L, P, R, and S	≥ 1 cDDD
Lai, 2013, Taiwan [36]	Case control	General population (NHIRD)	2000-2009	ICD-9-CM 155	1.4 years (M)	A, F, L, P, R, and S	≥1 Rx
Leung, 2013, Taiwan [37]	Case control	General population (NHIRD)	2000-2008	ICD-9-CM 155	4.1 years (M)	Unspecified	> 0.5 years
Chaiteerakij, 2013, USA [38]	Case control	Hyperlipidemia patients (Mayo Clinic)	2000-2010	ICD-9-CM 155	>1 years	Unspecified	≥1 Rx

Patients population: IPD = Individual patient data, RCT = randomized controlled trials, CRP = the Central Population Register of Danish citizens, KPMCP = the Kaiser Permanente Medical Care Program in northern California, GE Centricity = the General Electric Centricity database, NHIRD = the Taiwanese National Health Insurance research database, VISN = Veterans Integrated Service Networks 16 Veteran Affairs database, VA = Veterans Affairs national databases, Mayo Clinic = Mayo Clinic (Rochester, MN), HBV = hepatitis B virus; Cases defined: ICD-9 or -10 = International Classification of Diseases, Ninth Revision or Tenth Revision, CM = Clinical Modification; Duration of follow-up: When the follow-up periods of statin user and nonuser were different, only the shorter one was showed, and all periods were transformed to years; Statin type: A = Atorvastatin, C = Cerivastatin, F = Fluvastatin, L = Lovastatin, P = Pravastatin, R = Rosuvastatin, S = Simvastatin, Non-statin = Non-statin cholesterol-lowering drug(s) only; Duration of statin use: M = Mean, Me = Median, ≥1 cDDD = more than 1 cumulative defined daily dose before the diagnosis of liver cancer, Rx = prescriptions.

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Table 2. Study data

Studies	Intervention/ Cases		Control		Measurements of effect estimates	Crude RR with 95% CIs	Adjusted RR with 95% CIs	Confounders for adjustment
	No. of event/ No. of exposure	No. of total	No. of event/ No. of exposure	No. of total				
Emberson, 2012, UK [19]	35	67258	33	67279	RR	1.06 (0.66, 1.71)*	1.06 (0.66, 1.71)*	Randomization
Friis, 2005, North Jutland [28]	1	12251	166	334754	OR	NA	1.16 (0.46-2.90)	1,2, 16, 21, 23
Friedman(Male), 2008, USA [29]	32	192598	NA	1904876	HR	NA	0.49 (0.34-0.70)	16
Friedman(Female), 2008, USA [29]	10	169261	NA	1976332	HR	NA	0.40 (0.21-0.75)	
Marelli, 2011, USA [30]	13	45857	24	45857	RR	0.31 (0.14-0.68)*	0.31 (0.14-0.68)*	1-5, 14, 16-18, 26, 27
Tsan, 2012, Taiwan [31]	58	2785	963	30628	HR	0.66 (0.51- 0.86)	0.47 (0.36-0.61)	1, 2, 7, 8, 11, 12
Tsan, 2013, Taiwan [32]	1378	35023	26505	225841	HR	0.42 (0.39-0.46)	0.53 (0.49–0.58)	1, 2, 7, 8, 11, 13
Khurana, 2005, USA [33]	NA	NA	NA	NA	OR	NA	0.52 (0.41- 0.67)	1, 11, 13
El-Serag, 2009, USA [34]	447	1303	2766	5212	OR	0.46 (0.40-0.52)	0.74 (0.64-0.87)	1-3, 6, 8, 9, 11-13, 21, 24, 28
Chiu, 2011, Taiwan [35]	117	1166	195	1166	OR	0.53 (0.41-0.69)	0.62 (0.45-0.83)	1, 2, 8, 9, 11, 12, 20, 29
Lai, 2013, Taiwan [36]	255	3480	1635	13920	OR	0.61 (0.52–0.72)	0.71 (0.56–0.89)	1, 2, 8-13, 22, 24, 25
Leung, 2013, Taiwan [37]	26	424	6851	33781	HR	0.45 (0.30-0.67)	0.44 (0.28, 0.72)	1, 2, 11, 15, 20, 21, 23
Chaiteerakij, 2013, USA [38]	72	165	165	256	OR	NA	0.6 (0.4-0.9)	1-3, 8, 11, 17, 22, 28, 30

The RR with an asterisk mark (*) was calculated based on the raw data. The others, crude or adjusted, were extracted from the original paper; Confounders for adjustment: 1 = age, 2 = sex, 3 = race, 4 = BMI, 5 = smoking status, 6 = ethanol intake, 7 = socioeconomic status, 8 = cirrhosis, 9 = alcoholic liver disease, 10 = non-alcoholic fatty liver disease, 11 = diabetes mellitus, 12 = HBV infection, 13 = HCV infection, 14 = concomitant diagnoses (unspecified), 15 = Charlson score, 16 = calendar year, 17 = cholesterol (totalcholesterol, VLDL, LDL, or triglycerides), 18 = prostate-specific antigen, 19 = resection extent, 20 = other lipid-lowering agents, 21 = cardiovascular medications (aspirin, nonsteroidal anti-inflammatory medications, or angiotensin-converting enzymes inhibitors), 22 = metformin or thiazolidinedione, 23 = hormone-replacement therapy, 24 = HCV treatment, 25 = HBV treatment, 26 = medications taken (unspecified), 27 = the number of office visits, 28 = propensity to use statins, 29 = hospital stay, 30 = biliary tract diseases

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Table 3. Subgroup analyses of included studies

Subgroup		No. of studies (reports)	Summary RR (95% CIs)	Heterogeneity, I^2	Heterogeneity, *P* value	$P_{interaction}$
Study design	RCT	1	1.06 (0.66-1.71)	-	-	***P = 0.01***
	Observational studies	11(12)	0.57(0.50-0.64)	61%	***P* = 0.003**	
Observational studies	Cohort studies	5 (6)	0.51 (0.44–0.58)	18%	*P* = 0.30	***P* = 0.04**
	Case-control studies	6	0.63 (0.54–0.73)	46%	***P* = 0.10**	
Baseline risk of liver cancer	Higher baseline risk	4	0.52 (0.47-0.59)	16%	*P* = 0.31	***P*** = 0.08
	General population	8 (9)	0.63 (0.52–0.75)	59%	***P* = 0.01**	
Confounding adjustment	Adequate adjustment	6	0.64(0.53-0.77)	81%	***P* = 0.0001**	***P*** = 0.08
	Inadequate adjustment	6 (7)	0.51 (0.43-0.60)	3%	*P* = 0.40	
Study location	Western studies	8 (9)	0.61 (0.48-0.76)	64%	***P* = 0.007**	*P* = 0.54
	Asian studies	6	0.56 (0.48- 0.64)	51%	***P* = 0.09**	
Pharmacokinetic	Hipophilic statins	5 (6)	0.57 (0.50-0.65)	50%	***P* = 0.08**	***P*** = 0.86
	Hydrophilia statins	3	0.59(0.41–0.84)	50%	***P* = 0.13**	
Higher cumulative dosage of statin		6	0.53 (0.36-0.79)	90%	***P*<0.0001**	-

RR = relative risk; higher baseline risk of liver cancer: patients with older age, HBV or HCV infection. Adequate adjustment: RCT or studies which adjusted for at least 4 of 7 important confounders, such as HBV infection, HCV infection, cirrhosis, alcoholic liver disease, NAFLD, HBV treatment, or HCV treatment; Lipophilic statins: Atorvastatin, Fluvastatin, Lovastatin, or Simvastatin; Hydrophilia statins: Pravastatin or Rosuvastatin; Higher cumulative dosage of statin use: > 180cumulative defined daily dose or Duration of statin use > 0.5 years before the diagnosis of liver cancer.

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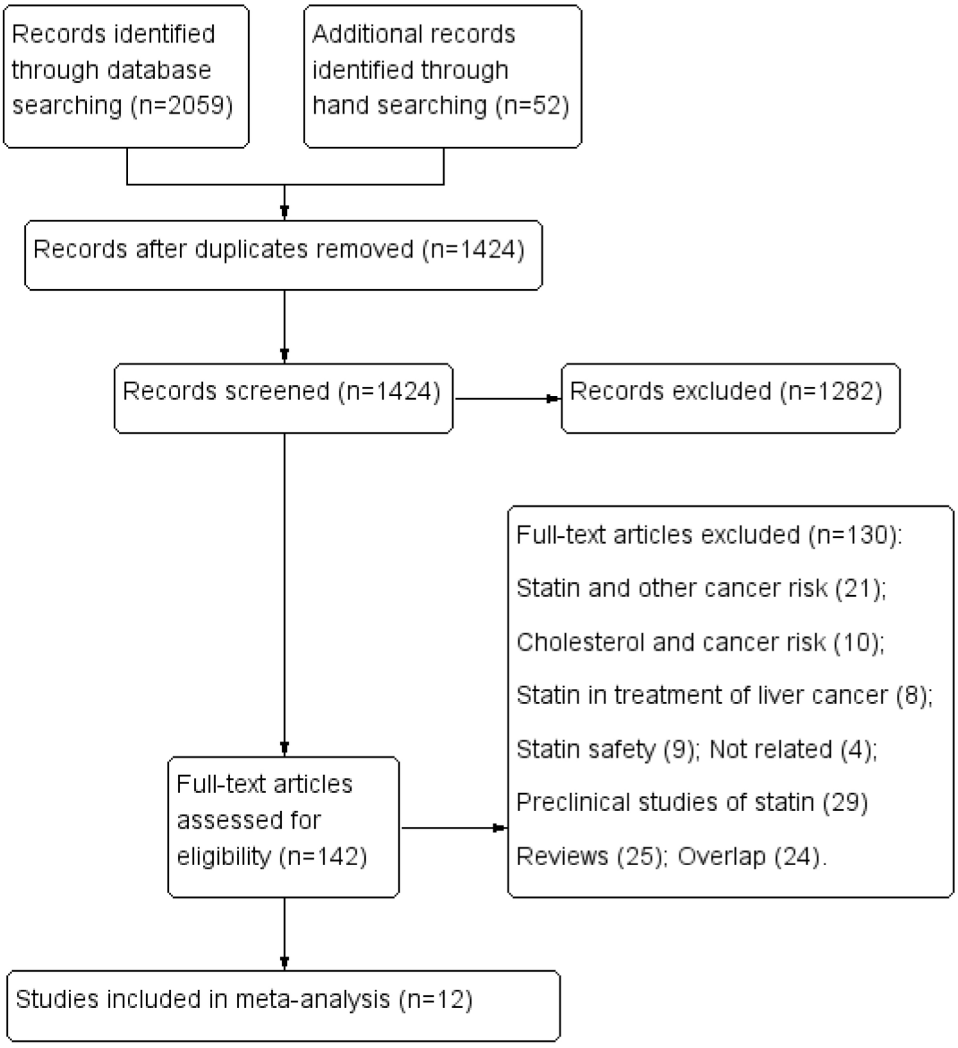

Flow chart of study selection in the present meta-analysis.
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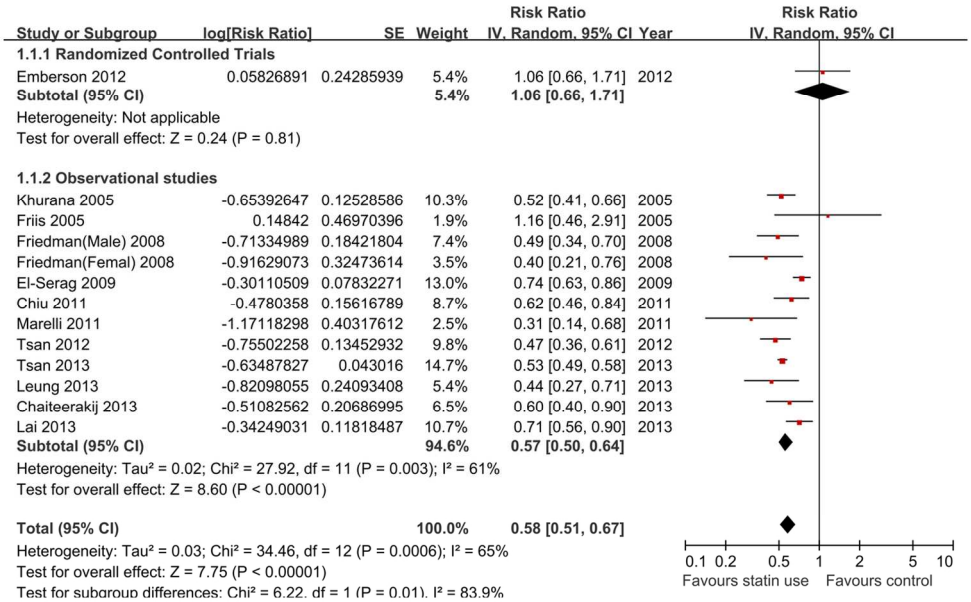

Overall meta-analysis of the statin use and the liver cancer risk.
128x80mm (300 x 300 DPI)

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SUPPLEMENTARY TABLES:

Supplementary Table 1. Assessment of methodological quality of the cohort and case-control studies according to the Newcastle–Ottawa Scale

Cohort Studies	Selection				Comparability	Outcome			Total Score
	Representativeness of the exposed cohort	Selection of the non-exposed cohort	Ascertainment of exposure	Outcome of present at start of study	Control for important factor	Assessment of outcome	Follow-up long enough	Adequacy of follow up	
Friis, 2005 [28]	☆	☆	☆	☆	☆	☆	-	☆	7
Friedman, 2008 [29]	☆	☆	☆	☆	☆	☆	-	☆	7
Marelli, 2011 [30]	☆	☆	☆	☆	☆	☆	☆	☆	8
Tsan, 2012 [31]	☆	☆	☆	☆	☆	☆	☆	☆	8
Tsan, 2013 [32]	☆	☆	☆	☆	☆	☆	☆	☆	8
Case–Control Studies	**Selection**				**Comparability**	**Exposure**			**Total Score**
	Adequate definition of cases	Representativeness of cases	Selection of controls	Definition of controls	Control for important factor	Ascertainment of Exposure	Same method for cases and controls	Non-response rate	
Khurana, 2005 [33]	-	☆	☆	☆	☆	☆	☆	-	6
El-Serag, 2009 [34]	-	☆	☆	☆	☆☆	☆	☆	-	7
Chiu, 2011 [35]	-	☆	☆	☆	☆☆	☆	☆	-	7
Lai, 2013 [36]	-	☆	☆	☆	☆☆	☆	☆	-	7
Leung, 2013 [37]	☆	☆	☆	☆	☆	☆	☆	☆	8
Chaiteerakij, 2013 [38]	-	☆	-	☆	☆	☆	☆	-	5

Control for important factor: ☆Reported relative risk have been adjusted for at least 4 of 7 important factors: HBV infection, HCV infection, cirrhosis, NAFLD, HCV treatment, HBV treatment, anti-diabetic medications; ☆ Study controls for any additional factor. Assessment of outcome: ☆ record linkage. Follow-up long enough: ☆ follow up period ≥ 4 years. Adequate definition of cases: ☆ The case is defined with independent validation. Non-response rate: ☆ Same rate for both groups.

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Supplementary Table 2. Studies reporting RR for use of lipophilic or hydrophilia statins, and for higher cumulative dosage of statin use

Studies	Measurements of effect estimates	Statins type	Dosage/Duration of Statin use	Crude RR with 95% CIs	Adjusted RR with 95% CIs
Tsan, 2012, Taiwan [31]	HR	A, F, L, P, R, and S	>365 cDDDs	0.50 (0.26-0.96)	0.34 (0.33-0.59)
	HR	Lipophilia statin	≥28 cDDDs	0.62 (0.47-0.83)	0.44 (0.33-0.59)
	HR	Hydrophilia statin	≥28 cDDDs	0.65 (0.39 -1.09)	0.51 (0.31-0.85)
Tsan, 2013, Taiwan [32]	HR	A, F, L, P, R, and S	>180 cDDDs	NA	0.33 (0.25–0.42)
El-Serag, 2009, USA [34]	OR	Simvastatin	1.6 years (M)	0.47 (0.41- 0.54)	0.64 (0.55-0.75)
Chiu, 2011, Taiwan [35]	OR	A, F, L, P, R, and S	>215.4 cDDDs	0.47 (0.30-0.72)	0.63 (0.37-1.06)
	OR	Lipophilia statin	≥ 1 cDDD	NA	0.56 (0.45–0.69)*
	OR	Hydrophilia statin	≥ 1 cDDD	NA	0.46 (0.29–0.71)*
Lai, 2013, Taiwan [36]	OR	Lipophilia statin	≥1 Rx	0.54 (0.48–0.61)*	0.67 (0.57–0.79)*
	OR	Hydrophilia statin	≥1 Rx	0.63 (0.47–0.83)*	0.80 (0.55–1.16)*

The RR with an asterisk mark (*) was calculated based on the raw data in the original study. The others, crude or adjusted, were extracted from the original paper.

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Supplementary Table 3. Published studies of the total cholesteroland the risk of liver cancer

Studies	Study design	cases/ participants	Follow-up	Reference (mg/dL)	Index (mg/dL)	Adjusted HR (95% CIs)		*P* for trend*	Confounders for adjustment
						Men	Women		
Iso, 2009, Japan [43]	Population-based cohort (JPHC Study)	125 /33,368	12.4 years	180–199	<160	2.62 (1.44–4.76)	4.15 (1.70–10.16)	Men < 0.0001 Women < 0.0001	1-10
					160–179	1.04 (0.52–2.07)	1.99 (0.82–4.85)		
					180–199	1	1		
					200–219	0.56 (0.24–1.28)	1.09 (0.44–2.68)		
					200–239	0.49 (0.16–1.44)	0.41 (0.11–1.52)		
					> 240	-	0.80 (0.28–2.27)		
Ahn, 2009, Finland [42]	Placebo-controlled, double-blinded primary prevention trial in male smokers (ATBC)	191/29,093	18.0 years	< 203.9	< 203.9	1	-	P=0.0007	1-5, 11-17
					203.9-227.6	0.69 (0.46-1.05)	-		
					227.7-249.2	0.63 (0.41-0.97)	-		
					249.3-276.6	0.56 (0.36-0.88)	-		
					> 276.7	0.66 (0.43-1.01)	-		
Kitahara, 2011, Korea [44]	Prospective study of Korean men and women (Korean NHIC)	10,161/1,189,719	12.7 years	< 160	< 160	1	-	Men < 0.001 Women < 0.001	2-5, 13, 18
					160-179	0.69 (0.65-74)	0.63 (0.54-0.72)		
					180-199	0.62 (0.58-0.66)	0.50 (0.44-0.58)		
					200-239	0.48 (0.45-0.51)	0.37(0.32-0.42)		
					≥ 240	0.42 (0.38-0.45)	0.32 (0.27-0.39)		

JPHC Study = The Japan Public Health Center-based Prospective Study, ATBC = The Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study, Korean NHIC = The Korean National Health Insurance Corporation Medical Evaluation. *Tests for linear trend were conducted by treating the total cholesterol as a continuous variable in the multivariable models. Confounders for adjustment: 1 = age, 2 = BMI, 3 = smoking, 4 = ethanol intake, 5 = hypertension, 6 = diabetes, 7 = hyperlipidemia medication use, 8 = total vegetable intake, 9 = coffee intake, 10 = public health center, 11 = intervention, 12 = level of education, 13 = physical activity, 14 = Saturates fat intake, 15 = polyunsaturated fat intake, 16 = total calorie, 17 = serum HDL cholesterol, 18 = fasting serum glucose.

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Supplementary Table 4. Published trials of statin use as adjuvant in treatment of liver cancer

Studies	Study design	Patients population	Intervention	Control	Overall survival of intervention (months)	Overall survival of control (months)	Kaplan-Meier and log-rank test
Kawata, 2001, Japan [49]	Prospective, randomized, open label study	Patients with advanced liver cancer after TAE procedure, n = 83	Pravastatin 20-40 mg + 5-FU 200 mg QD, n = 41	5-FU 200 mg QD, n = 42	Median 18	Median 9	*P* = 0.006
Lersch, 2004, Germany [50]	Prospective study	Patients with advanced liver cancer, n = 58	Pravastatin 40-80 mg QD, n = 20	A: Octreotide, n = 30; B: Gemcitabine, n = 8	Median 7.2 (95% CIs 2.9-11.5)	A: Median 5(95% CIs 2.2-7.8);B: Median 3.5 (95% CIs 2.2-4.9)	A: *P* = 0.09; B: *P* = 0.03
Graf, 2008, Germany [51]	Prospective, non-randomized, open label study	Patients with advanced liver cancer after TACE, n = 183	Pravastatin 20-40 mg QD, n = 52	No treatment, n = 131	Median 20.9 (95% CIs 15.5-26.3)	Median 20.9 (95% CIs 15.5-26.3)	*P* = 0.003
Georgescu, 2011,Romania [52]	Prospective, randomized, open label study	Patients with advanced liver cancer, n = 72	Lovastatin 40 mg + Sorafenib 400 mg QD, n = 39	Sorafenib 400 mg QD, n = 33	Mean 12.15±0.76	Mean 10.85±0.82	Non-significant

TAE = Transcatheter Arterial Embolization; TACE = Transhepatic Arterial Chemotherapy and Embolization).

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Supplementary Table 5. Ongoing clinical trials of statin use as adjuvant in treatment of liver cancer

Studies	Year	Location	Phase	Study design	Condition	Intervention	Control	Estimated Enrollment	Resist number	Status
ESTAHEP-2010	2011	Spain	II	Multicenter, prospective, randomized, double-blind, placebo-controlled study	Advanced liver cancer	Sorafenib 400 mg BID + Pravastatin 40 mg, QD	Sorafenib 400 mg BID + placebo QD	216	NCT01418729; EUCTR2010-024421-21-ES	Recruiting
PRODIGE 21	2011	France	II	Multicenter, prospective, randomized, open label study	Liver cancer with Child-Pugh B Cirrhosis	A: Sorafenib 400 mg BID;B: Pravastatin 40 mg, QD;C: Sorafenib 400 mg BID + Pravastatin 40 mg, QD	Best supportive care	160	NCT01357486	Recruiting
JOUVE PHRCK 2009	2013	France	III	prospective, randomized, open label study	Liver cancer with Child-Pugh A Cirrhosis	Sorafenib 800 mg BID + Pravastatin 40 mg, QD	Sorafenib 800 mg BID	474	NCT01903694; NCT01075555	Recruiting

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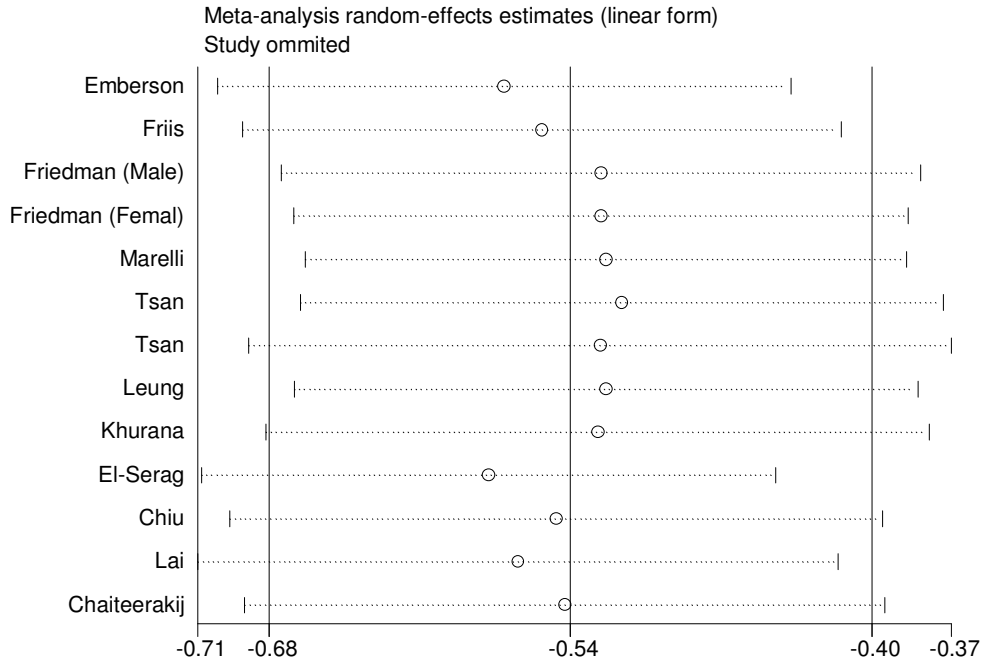
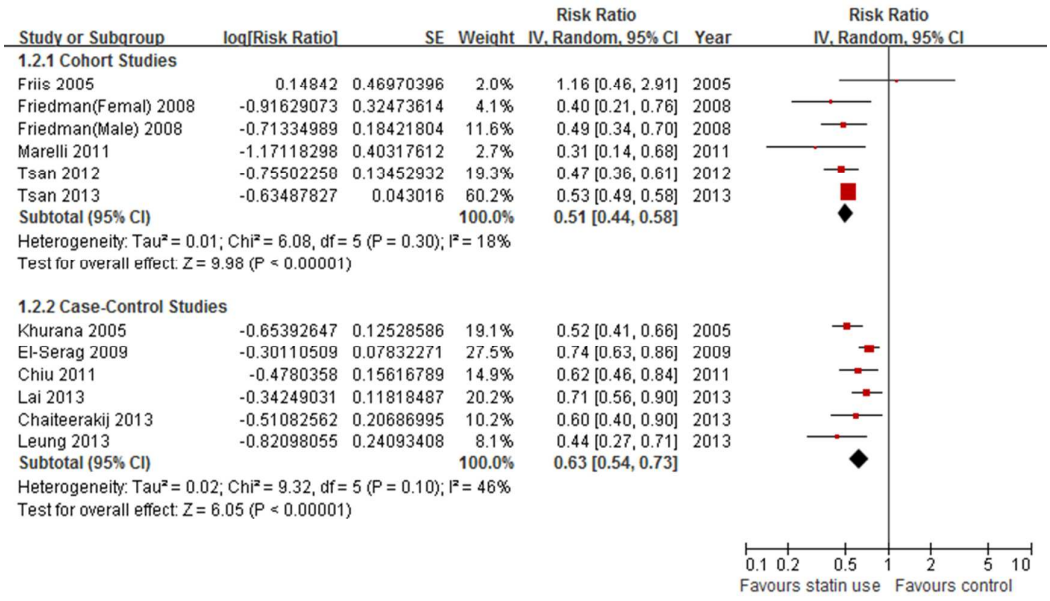
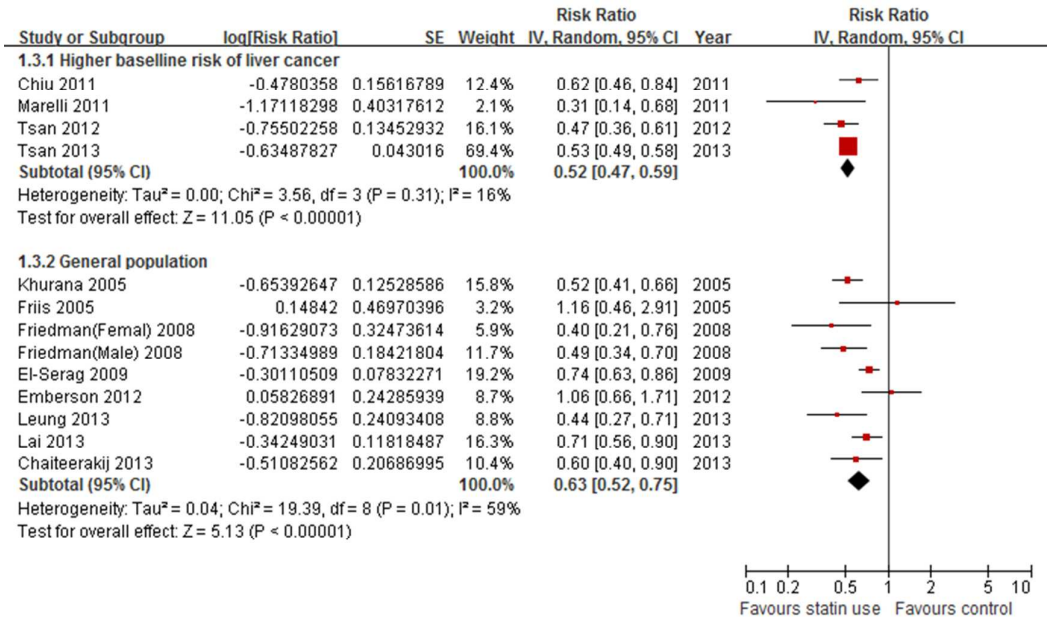
SUPPLEMENTARY FIGURES:



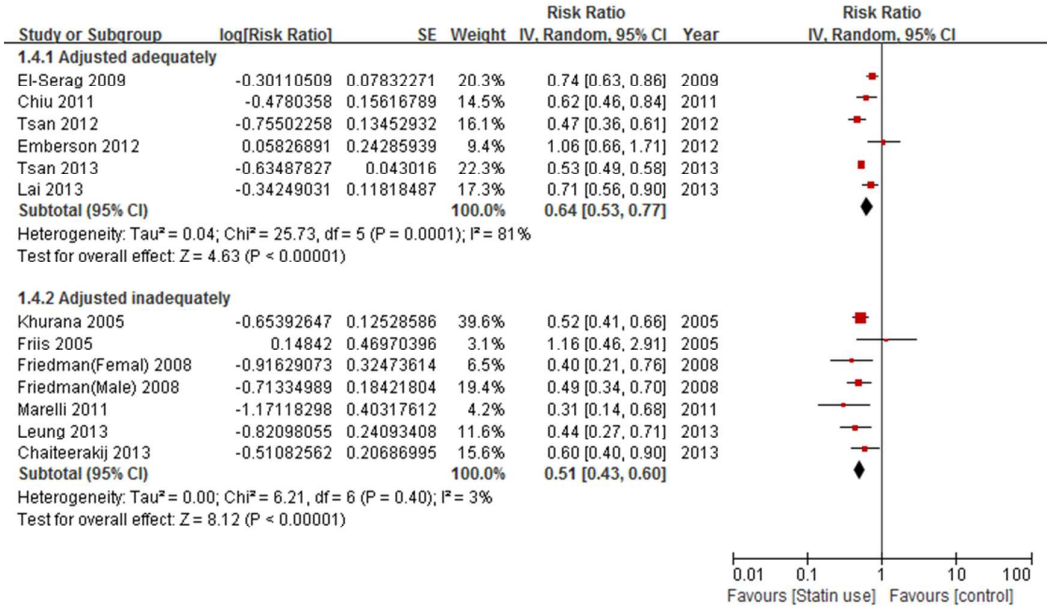
Supplementary Figure 1. Influence analysis.



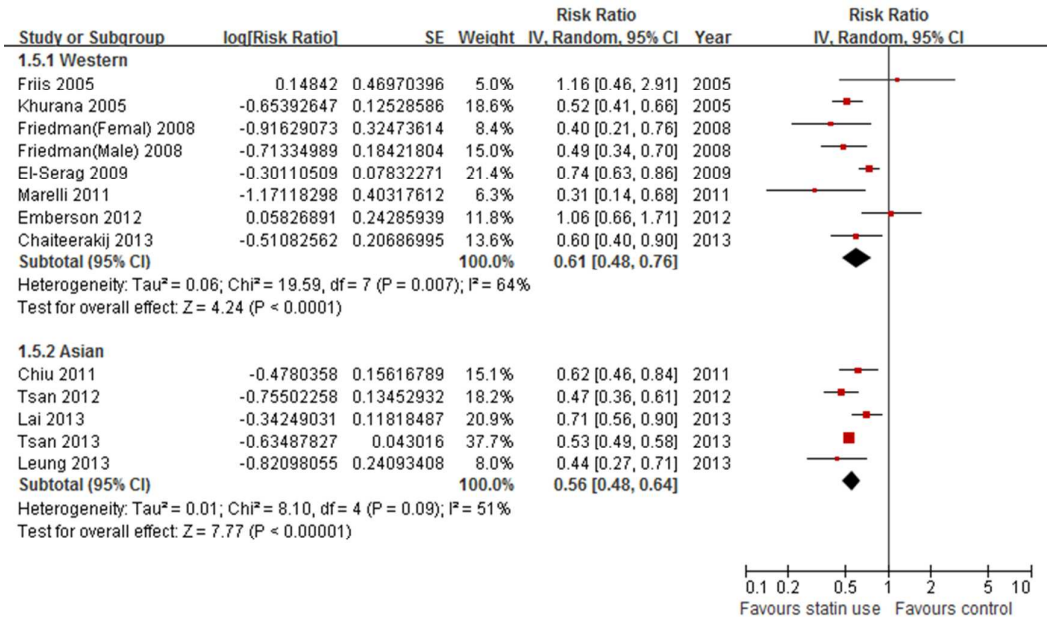
Supplementary Figure 2. Subgroup analyses based on study design.



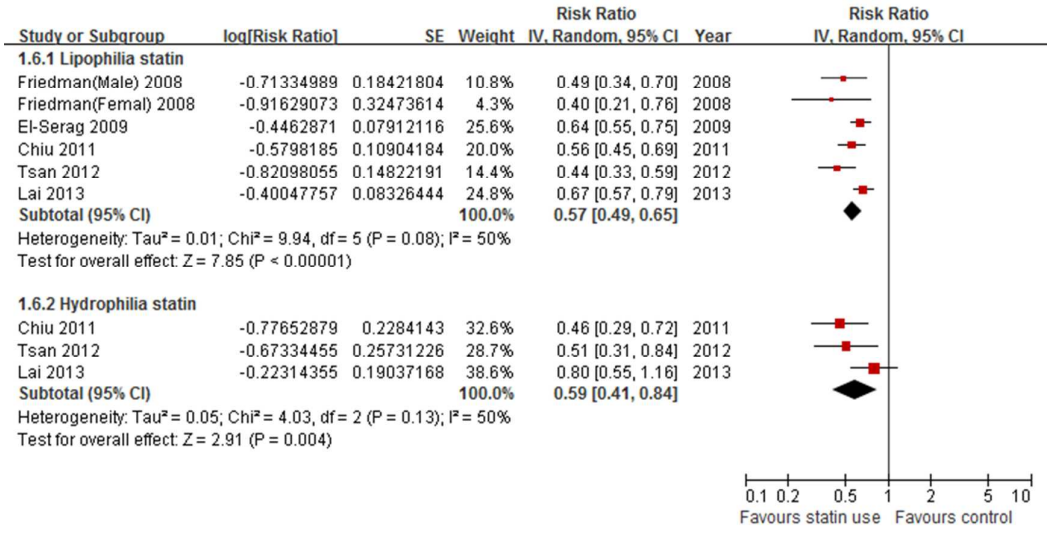
Supplementary Figure 3. Subgroup analyses based on baseline risk of liver cancer.



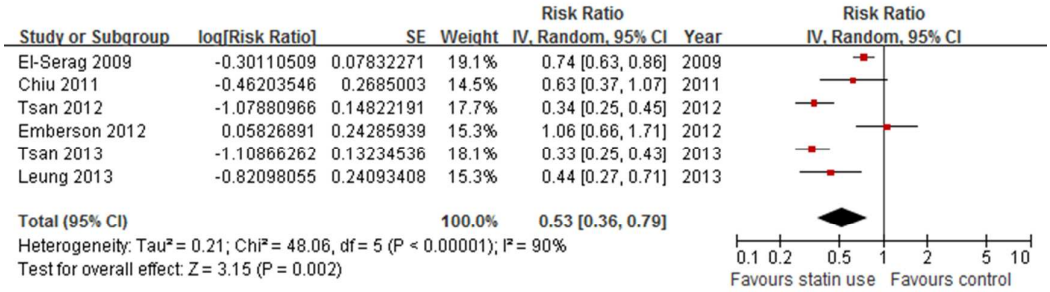
Supplementary Figure 4. Subgroup analyses based on confounder adjustment.



Supplementary Figure 5. Subgroup analyses based on study location.



Supplementary Figure 6. Subgroup analyses based on pharmacokinetic of statins.



Supplementary Figure 7. Subgroup analysis of higher cumulative dose of statin use.

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	4
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	5
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	no
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	6
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	5
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	5
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	6
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	6-7
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	6-7
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	7
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	7
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	7

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PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	8
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	8
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	8
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	9 Table1,2
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	10, Suppl. Table 1
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	Figure 2
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	10
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	10
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	10-12
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	12-13
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	13-14
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	15-16
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	NA

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(6): e1000097. doi:10.1371/journal.pmed1000097

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Statin use and risk of liver cancer: an update meta-analysis

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Statin use and risk of liver cancer: an update meta-analysis

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Running title: Meta-analysis: statin and liver cancer

Key words: *Statin; Liver cancer; Cancer Prevention; Meta-analysis.*

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Abstract

Objective: Statins are commonly prescribed cholesterol-lowering drugs. Preclinical studies suggest that statins may possess cancer preventive properties. The primary objective of this meta-analysis was to determine the association between the statin use and the risk of liver cancer.

Design: Meta-analysis.

Setting: International.

Participants: A comprehensive literature search of PubMed, BIOSIS Previews, Web of Science, EMBASE, EBSCO and Cochrane Library was conducted through March 2014. The effect estimate was reported as pooled relative risk (RR) with 95% confidence intervals (CIs), using the random-effects model.

Results: A total of 12 studies (one individual patient data analysis of 22 randomized controlled trials, 5 cohorts, and 6 case-controls) were qualified for this meta-analysis, involving 5,640,313 participants including 35,756 liver cancer cases. Our results indicated a significant risk reduction of liver cancer among all statin users (RR 0.58, 95%CIs 0.51–0.67). The difference of the study designs can partly explained the significant heterogeneity found in the overall analysis (I^2 = 65%, P = 0.0006). No evidence of publication bias was observed in this meta-analysis. Similar risk reductions were found in the subgroups analysis of Western and Asian countries, lipophilic and hydrophilia statins. There was a trend toward more risk reductions in subgroups with higher baseline risk, inadequate adjustment, and higher cumulative dosage of statin use.

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Conclusions: This meta-analysis suggests that the statin is associated with a significant risk reduction of liver cancer, when taken daily for cardiovascular event prevention. However, this preventive effect might be overestimated due to the exposure period, tthe indication and contraindication of statins, and other confounders. Statins might be considered as an adjuvant in the treatment of liver cancer.

Strengths and limitations of this study

Statins are commonly prescribed as cholesterol-lowering drugs. In this comprehensive meta-analysis, we demonstrate that the statin use is associated with a significant risk reduction of liver cancer.

The difference of the study designs is the part reason that explained the significant heterogeneity found in the overall analysis.

However, this preventive effect might be overestimated due to the exposure period, tthe indication and contraindication of statins, and other confounders.

Statins might be considered as an adjuvant in the treatment of liver cancer.

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Introduction

Statins are inhibitors of 3-hydroxy-3-methylglutaryl coenzyme-A (HMG-CoA) reductase and they are widely used to reduce the plasma cholesterol level and the risk of cardiovascular events.[1] Although there is a concern over their possible carcinogenicity raised in rodent studies,[2] preclinical studies indicate that statins have anticancer properties *in vitro* and *in vivo*, through inhibiting angiogenesis, inducing apoptosis, and suppressing tumor growth and metastasis.[3-5]

However, higher concentrations of statins are typically required to induce these effects, raising questions concerning the therapeutic relevance of statins on cancer.[6] To date, clinical studies regarding the cancer incidence associated with statin administration have highlighted conflicting results. Moreover, a large number of meta-analyses have concluded that there was no association between statin use and risk of overall cancer,[7-10] or cancer of breast[11], stomach,[12] or pancreas.[13] There is only a modest protective effect of statins in prostate cancer[14] and colorectal cancer.[15]

In contrary, recent studies reported encouraging results for risk reduction of liver cancer among all statin users. Previous meta-analysis, conducted by Singh *et al*. by including 10 studies, found that statin users were less likely to develop hepatocellular carcinoma (HCC) than statin non-users.[16] However, Singh *et al*. included the ALERT, LIPS, and MEGA trials twice, by including three individual patient data (IPD) analysis of randomized controlled trials (RCTs).[17-19] Meanwhile, some factors of stratification were not considered in their analyses, such as dose and timing of exposure to statins, and the selection of controls and confounders, which might limit

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the evaluation of cancer risk.[20] Furthermore, the lipophilic statins are accompanied by an extensive first-pass effect at the hepatic level.[21] It is plausible that lipophilic statins may have a better liver cancer preventive qualities than the hydrophilic ones.[22]

Therefore, we performed this updated meta-analysis to assess the association between the statin use and the risk of liver cancer, involving the recently published studies and conducting more subgroup analyses based on the factors mentioned above. Our results demonstrated that statin use was associated with an over 40% risk reduction in liver cancer, which may have a significant translational potential in the clinical practice. However, there were some confounders might overestimate this preventive effect of statins.

MATERIALS AND METHODS

Literature Search strategy

This meta-analysis was conducted following the PRISMA guidelines.[23]

The systematic computerized search for eligible studies were performed on the database of PubMed, BIOSIS Previews, Web of Science, EMBASE, EBSCO, and Cochrane Library, covering all studies published from their inception to March 5, 2014. The following terms were searched with both the subjects (MeSH terms) and text-word search strategies: "(Statin OR HMG-CoA reductase inhibitors OR Atorvastatin OR Cerivastatin OR Fluvastatin OR Lovastatin OR Pravastatin OR Rosuvastatin OR Simvastatin) AND (Hepatocellular OR Hepatic OR Intrahepatic OR Interlobular OR Liver) AND (Carcinoma OR Sarcomas OR Angiosarcoma OR Cancer OR Neoplasm). Additionally, the relevant reviews and retrieved articles were searched

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manually for more eligible studies.

In study searching, only the original researches, published in form of peer review article or meeting abstract, were included. No language restrictions were imposed. However, the studies we included were all published in English.

Study selection

The inclusion criteria were: (1) randomized controlled trial (RCTs), cohort studies or case-control studies; (2) original studies that assessed the effect of statin use on the risk of liver cancer, compared with placebo or no treatment; (3) liver cancer cases were identified according to the International Classification of Diseases codes (ICD); and (4) studies with estimate of relative risk (risk ratio, RR) of liver cancer, or with data sufficient to calculate it.

The exclusion criteria were: (1) study design not meeting the inclusion criteria; (2) studies without estimate of RR, or without sufficient data to calculate it; or (3) studies with duplicated or overlap reports.

Data extraction

Two independent investigators (M. Shi and X.B. Cui) extracted data from the eligible studies using a predefined data collection form. The differences of data extraction were resolved by consensus referring back to the original article. The extracted information included: (1) Studies: first author, year of publication, study design, location, patient populations, period, and follow-up; (2) Statins: type, dosage or duration of statin use; (3) liver cancer: case identification, number of liver cancer, crude RR with 95% confidence intervals (CIs), adjusted RR reflecting the greatest

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degree of control for confounders, and confounders for adjustment (including variables for matching). When the RR were not available, the RR with 95% CIs were calculated from the raw data in original studies.

We extracted different measurements of effect estimates from original studies, such as Relative Risk (RR), Odds Ratio (OR), Hazard Ratio (HR), and Observed/Expected ratio. Due to the fact that the incidence of liver cancer was low in all studies, theses different measurements can be used to provide similar estimates of RR.

Methodological quality assessment

Of note, the included RCT was pooled analysis of other RCTs, therefore, it is inappropriate to assess the methodological quality. The methodological quality of cohort and case-control studies were assessed on the Newcastle-Ottawa Scale,[24] including eight items that were categorized three categories: selection (four items, one star each), comparability (one item, up to two stars), and exposure/outcome (three items, one star each). A "star" presents a "high" quality choice of each item.

Statistical analysis

The overall meta-analysis was first performed, followed by the subgroup analyses, based on study design, baseline risk of liver cancer, confounding adjustment, study location, and pharmacokinetic. Meanwhile, we conducted subgroup analyses based on studies which reported RR estimate for higher cumulative dosage of statin use, when appropriate data were available.

To take into account the heterogeneity and provide a more conservative estimate, the inverse variance method was used to estimate the pooled RR and corresponding 95%

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CIs, and data were pooled using a random effects model. Heterogeneity was assessed using the Chi-squared statistic (P) together with the Higgins I-squared statistic (I^2), a P value <0.10 was considered statistically significant for heterogeneity; and an I^2 value > 50 % was considered a measure of severe heterogeneity.[25]

Publication bias was assessed using the Begg's test and the Egger's test.[26] Influence analysis was performed to investigate the influence of a single study on the overall meta-analysis estimate, by omitting one study in each turn. Test for interaction was applied to identify the difference between pooled RR from subgroup analysis using the method described by Altman and Bland.[27] All statistical tests were two-sided and $P < 0.05$ was considered statistically significant, unless otherwise specified. Software Review Manager (RevMan5.2, Copenhagen) and STATA (Stata 11.2, Texas) were used for the statistical analysis.

Results

Study selection

Figure 1 illustrated the process of study selection for the meta-analysis. Of the 1424 potentially relevant references identified by electric and manual search, 142 were selected for full-text review after screening titles and abstracts. Finally, a total of 12 studies were included, with one IPD analysis,[19] five cohort studies,[28-32] and six case-control studies.[33-38] One case-control study was presented solely in abstract form.[33]

Of note, the cohort study conducted by Friedman *et al.* reported RR estimate separately for different gender (male and female),[29] we considered these two reports

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as separate studies. Therefore, a total of thirteen reports were included for the present meta-analysis.

Study characteristics

Table 1 summarized the characteristics of qualified studies in this meta-analysis. The 12 studies, involving 5,640,313 participants with 35,756 liver cancer cases, were published between 2005 and 2013. The "RCT" in the present study was pooled analysis of 22 clinical trials,[19] which investigated statins therapy in cardiovascular event prevention and reported the occurrence of liver cancer as adverse event. The observational studies were conducted with the local or national health databases, the statin exposure were identified by linkage to prescription databases, and the controls were matched mainly by age, sex and index date. Except one cohort adopted ICD-10 C22,[28] all other studies identified liver cancer cases according to the ICD-9 155. Of note, two cohorts were restricted to patients with HBV infection,[31] and HCV infection;[32] one case-control only included patients with diabetes mellitus;[34] two observational studies included patients aged at least 45 years.[30 35]

Table 2 summarized the data of the included studies. In the RCT[19] and one cohort study,[30] the RR with 95% CIs were calculated from the 2×2 tables defined by the incidence of liver cancer and the statin use status. The observational studies reported different measurements of RR estimates with adjustment by confounders. Several observational studies adopted the important risk factors of liver cancer for adjustments[31 32 34-36], such as HBV infection, HCV infection, cirrhosis, alcoholic liver disease, or non-alcoholic fatty liver disease (NAFLD).[39] Of note, only two studies

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adjusted for the cholesterol level,[30 38] and no study adjusted for the metabolic syndrome, which might also influence the risk of liver cancer.[39]

Methodological quality

For the cohort and case-control studies, the median score was 7 on the Newcastle-Ottawa Scale, with a range of 5 to 8 (**Supplementary Table 1**). These results indicated that the observational studies were in a reasonable good quality.

Overall meta-analysis

Figure 2 depicted the forest plot of RR estimate with 95% CIs from individual studies and overall meta-analysis. In the overall meta-analysis, pooled results showed a statistically significant decrease in the liver cancer risk among all statin users (RR 0.58, 95%CIs 0.51–0.67). Of note, a statistically significant heterogeneity was observed (I^2 = 65%, P = 0.0006). The P-values of Begg's test and Egger's test were 0.669 and 0.749, respectively, both suggesting there was no evidence of publication bias. In the influence analysis, the omission of any individual studies did not alter the direction and magnitude of the observed effect (**Supplementary Figure 1**).

Subgroup analyses and Test for interaction

We first performed preplanned subgroup analyses based on study design, baseline risk of liver cancer, confounding adjustment, and study location (**Table 3**).

The RCT showed there is no significant association between statin use and risk of liver cancer (RR 1.06, 0.66–1.71). But the observational studies indicated a significant decrease of liver cancer risk among all statin users (RR 0.57, 0.50–0.64; I^2 = 61%, P = 0.003) (**Figure 2**). Furthermore, we found a greater risk reduction in the subgroup

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analysis of cohort studies (RR 0.51, 0.44–0.58; I^2 = 18%, P = 0.30) than in the case-control studies (RR 0.63, 0.54–0.73; I^2 = 46%, P = 0.10) (**Supplementary Figure 2**).

Test for interaction showed significant results between subgroups of the RCT and observational studies ($P_{interaction}$ = 0.01, Z = 2.47), and between subgroups of the cohort and case-control studies ($P_{interaction}$ = 0.04, Z = -2.03). These results indicated that the difference of the study designs was the part reason that why there was severe heterogeneity in the overall analysis (**Table 3**).

In the subgroup analysis of the four studies with higher baseline risk of liver cancer,[30-32 35] defined as patients with older age, HBV or HCV infection, there was a trend toward more decrease of liver cancer risk (RR 0.52, 0.47-0.59; I^2 = 16%, P = 0.31) than in the other eight studies with general population[19 28 29 33 34 36-38] (RR 0.63, 0.52–0.75; I^2 = 59%, P = 0.01) (**Supplementary Figure 3**).

We defined the RCT or studies adjusted for at least 4 of 7 important confounders, such as HBV infection, HCV infection, cirrhosis, alcoholic liver disease, NAFLD, HBV treatment, or HCV treatment,[39] were adjusted adequately. Subgroup analysis of these six studies[19 31 32 34-36] found a trend toward less decrease of liver cancer risk (RR 0.64, 0.53-0.77; I^2 = 81% P = 0.0001) than the other six studies[28-30 33 37 38] (RR, 0.51, 0.43-0.60; I^2 = 3%, P = 0.40) (**Supplementary Figure 4**).

Subgroup analyses based on study location found a similar risk reduction of liver cancer in the Western countries (RR 0.61, 0.48–0.76; I^2 = 64%, P = 0.007) and in the Asian countries (RR 0.56, 0.48–0.64; I^2 = 51%, P = 0.09). (**Supplementary Figure 5**)

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Besides the overall RR estimates, some studies reported different RR estimate for different pharmacokinetic and dosage of statin use (**Supplementary Table 2**). We conducted further subgroup analyses based on these available data.

According to the different pharmacokinetic, statins can be classified as lipophilic statins (Atorvastatin, Fluvastatin, Lovastatin, and Simvastatin) and hydrophilia statins (Pravastatin and Rosuvastatin).[21] Subgroup analysis of lipophilic statins [29 31 34-36] found a significant decrease of liver cancer risk (RR 0.57, 0.50–0.65; I^2 = 50%, P = 0.08). And there was a similar result among users of hydrophilia statins[31 35 36] (RR 0.59, 0.41–0.84; I^2 = 50%, P = 0.13) (**Supplementary Figure 6**).

Test for interaction showed non-significant results for subgroups with different baseline risk, confounding adjustment, study location, or pharmacokinetic ($P_{interaction}$ = 0.08, 0.08, 0.54 and 0.86, respectively) (**Table 3**). Therefore, there is no strong evidence to support a different preventive effect of statins on liver cancer in these subgroups.

Subgroup analysis of six studies with higher cumulative dose of statin use, defined as statin use more than 180 cumulative defined daily dose (cDDDs) or 0.5 years (cumulative duration), showed a trend toward more risk reduction of liver cancer (RR 0.53, 0.36-0.79), but with a high degree of heterogeneity (I^2 = 90%, P<0.00001) (**Supplementary Figure 7**).

Discussion

This present meta-analysis represents the most comprehensive review to date on the association between the statin use and the liver cancer risk, by including 12 studies

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(one IPD analysis of 22 RCTs, 5 cohort studies, and 6 case-control studies) and involving 5,640,313 participants with 35,756 liver cancer cases. Overall, we found that statin use was associated with an over 40% risk reduction in liver cancer compared with nonusers (RR 0.58, 95%CIs0.51–0.67). This result was in line with the previous three meta-analyses: Singh *et al.* included 10 studies and suggested statin users were less likely to develop HCC (OR 0.63, 95%CIs 0.52-0.76),[16] Pradelli *et al.* and Zhang *et al.* included 5 and 7 observational studies and found a summary RR of 0.58 (95%CIs 0.46–0.74) and 0.61 (95%CIs 0.49–0.76), respectively.[40 41]

The IPD analysis of 22 RCTs showed there is no significant association between statin use and risk of liver cancer. The significant risk reduction of liver cancer among all statin users was seen primarily in the observational studies, and this preventive effect was relatively convinced in the cohorts than in the case-controls. There were some reasons to explain the different findings between RCTs and observational studies.

First, the exposure period to statins might be shorter than the period to carcinogenesis and the latency to diagnosis in the cohorts and the case-controls. The observational studies defined statin use varying in dosage and duration, from patients who received ≥1 cDDD or >1 Rx of statins to more than 0.5 years (**Table1**). On the other hand, the median period of statin use was 5.1 years in the RCTs. Although there was a trend toward more risk reduction of liver cancer with higher cumulative dose of statin use, this defect might still result in overestimating the cancer-preventive effect of statins in the observational studies.

Second, clinical studies demonstrated that higher serum total cholesterol

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concentration was associated with decreased risk of liver cancer (**Supplementary Table 3**).[42-44] Meanwhile, there were inverse association between use of non-statin lipid-lowering drugs and risk of the liver cancer.[35 38] Meanwhile, because of the contraindication, statins might not prescribed to the patients with the chronic liver disease, which is known as a risk factor of liver cancer. Unfortunately, the observational studies included in this analysis seldom adopted these factors for adjustment. Actually, subgroup analysis of studies with adequate adjustment showed a trend toward less risk reduction, indicating the potential of overestimate this preventive effect by confounders.

Third, the RCTs included lower risk population (patients with cardiovascular disease rather than HBV /HCV infection), might not be powerful enough to investigate the liver cancer outcomes, which were much rarer than cardiovascular events. In addition, subgroup analysis of studies with higher baseline risk showed a trend toward more decrease of liver cancer risk.

These reasons suggested that the observed modulation of cancer incidence cannot be ascribable to a direct statin-mediated effect,[20] the exposure period, the indication (e.g. hyperlipidemia) and contraindication (e.g. chronic liver disease) of statins might overestimate its cancer-preventive effect.

We found similar results in Western countries and Asian countries, which were different from the meta-analysis conducted by Singh *et al.* which concluded that the inverse association of statins with HCC was stronger in the Asian population. Considering four more studies we included, this difference might be caused by the

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insufficient data in their meta-analysis. Based on the pharmacokinetics, it is plausible that lipophilic and hydrophilic statins will differ in their liver cancer prevention qualities.[21 22] However, subgroup analysis of lipophilic and hydrophilic statins showed similar results.

Besides the limitations described previously, there were some other limitations should be noted. First, a significant heterogeneity was observed in the present meta-analysis, which might results from the difference in study design. Results of subgroup analyses would also be limited by this heterogeneity. Second, the adherence to statin therapy is known to be associated with healthy lifestyle, which might affect the cancer outcome.[45] Such information is hard to be captured in databases or medical record in the observational studies.[46] Third, five observational studies were conducted using the Taiwanese National Health Insurance Research Database (NHIRD),[31 32 35-37] although they were not in the same period, these studies might contain overlapping groups of patients. These limitations mentioned above might lead to confounding of overall results from the present study, and should be considered in future studies aiming at confirming the protective effects of statins on human cancer risk.

The strengths of our meta-analysis were as follows: First, we performed a much more comprehensive search and more subgroup analyses, compared with the previous meta-analyses; Second, the methodological quality of the included studies were reasonable good; Third, publication bias, which due to the tendency of not publishing small studies with null results, were not found in our meta-analysis.

Of note, preclinical studies have indicated that statins possess synergism with other

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therapeutic agents *in vitro* and *in vivo* for liver cancer.[47 48] Some clinical studies have also demonstrated that statins would prolong survival in patients with advanced liver cancer (**Supplementary Table 4**),[49-52] and associated with risk reduction of recurrence after curative surgery in patients of HBV related HCC.[53] Therefore, considerable interest exists in adjunctive therapy with statins for liver cancer. In fact, there were some RCTs ongoing to determine the effectiveness of pravastatin, when used in combination with sorafenib, in the treatment of liver cancer (**Supplementary Table 5**).

Currently, physicians are less likely to prescribe statins for patients with chronic liver disease, based on the concerns about the statin-induced liver injury.[31] However, there were number of studies have demonstrated the safe use, even salutary effects.[54-56] Meanwhile, the risk of serious statin-related liver injury appears to be no greater than the background incidence of this rare event.[57] Therefore, considering their benefits for cardiovascular event prevention and the potential effect in liver cancer prevention and treatment, statins should not be denied to these patients.

In conclusion, our results suggest that statin use is associated with a significant risk reduction of liver cancer, when taken daily for cardiovascular event prevention. However, this preventive effect might be overestimated due to the exposure period, indication and contraindication of statins, and other confounders. Statins might be considered as an adjuvant in the treatment of liver cancer.

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CONTRIBUTORSHIP STATEMENT

XB Cui had the original idea, M Shi, XB Cui and W Gong worked together to develop an appropriate theoretical framework and design. XB Cui developed the search, M Shi and XB Cui were involved in the selection process. M Shi and XB Cui extracted relevant data, XB Cui and W Gong performed the statistical analysis and all authors were involved in the data interpretation. M Shi and B Nie wrote the manuscript draft and revised the draft based on input from the other authors. All authors revised it critically for content and approved the final version.

COMPETING INTERESTS

There are no competing interests

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No additional data available.

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Figure legends:

Figure 1. Flow chart of study selection in the present meta-analysis.

Figure 2. Overall meta-analysis of the statin use and the liver cancer risk.

Supplementary Figure 1. Influence analysis.

Supplementary Figure 2. Subgroup analyses based on study design.

Supplementary Figure 3. Subgroup analyses based on baseline risk of liver cancer.

Supplementary Figure 4. Subgroup analyses based on confounder adjustment.

Supplementary Figure 5. Subgroup analyses based on study location.

Supplementary Figure 6. Subgroup analyses based on pharmacokinetic of statins.

Supplementary Figure 7. Subgroup analysis of higher cumulative dose of statin use.

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Table 1. Study characteristics

Studies	Study design	Patient population	Study period	Cases defined	Follow-up	Statins type	Dosage/Duration of Statin use
Emberson, 2012, UK [19]	RCT	IPD analysis of 22 RCTs	-	ICD-9 155	5.1 years (Me)	A, F, L, P, R, S	5.1 years (Me)
Friis, 2005, North Jutland [28]	Cohort	General population (CPR)	1989-2002	ICD-10 C22	3.3 years (M)	Unspecified	≥2 Rx
Friedman, 2008, USA [29]	Cohort	General population (KPMCP)	1994-2003	ICD-9-CM 155	> 2 years	A, L, S (97.6%)	≥1 Rx
Marelli, 2011, USA [30]	Cohort	General older population (men ≥ 45 and women ≥ 55 years; GE Centricity)	1990-2009	ICD-9 155	4.6 years (M)	Unspecified	≥1 cDDD
Tsan, 2012, Taiwan [31]	Cohort	Patients with HBV infection (NHIRD)	1997-2008	ICD-9 155	9.9 years (M)	A, F, L, P, R, and S	≥28 cDDDs
Tsan, 2013, Taiwan [32]	Cohort	Patients with HCV infection (NHIRD)	1999-2010	ICD-9 155	10.7 years (M)	A, F, L, P, R, and S	≥28 cDDDs
Khurana, 2005, USA [33]	Case control	General population (VISN)	1997-2002	ICD-9 155	NR	Unspecified	≥1 Rx
El-Serag, 2009, USA [34]	Case control	Diabetes patients (VA)	1997-2002	ICD-9-CM 155	2.4 years (M)	A, C, F, L, P, and S	1.6 years (M)
Chiu, 2011, Taiwan [35]	Case control	Older patients(≥ 50 years; NHIRD)	2005–2008	ICD-9-CM 155	NR	A, F, L, P, R, and S	≥ 1 cDDD
Lai, 2013, Taiwan [36]	Case control	General population (NHIRD)	2000-2009	ICD-9-CM 155	1.4 years (M)	A, F, L, P, R, and S	≥1 Rx
Leung, 2013, Taiwan [37]	Case control	General population (NHIRD)	2000-2008	ICD-9-CM 155	4.1 years (M)	Unspecified	> 0.5 years
Chaiteerakij, 2013, USA [38]	Case control	Hyperlipidemia patients (Mayo Clinic)	2000-2010	ICD-9-CM 155	>1 years	Unspecified	≥1 Rx

Patients population: IPD = Individual patient data, RCT = randomized controlled trials, CRP = the Central Population Register of Danish citizens, KPMCP = the Kaiser Permanente Medical Care Program in northern California, GE Centricity = the General Electric Centricity database, NHIRD = the Taiwanese National Health Insurance research database, VISN = Veterans Integrated Service Networks 16 Veteran Affairs database, VA = Veterans Affairs national databases, Mayo Clinic = Mayo Clinic (Rochester, MN), HBV = hepatitis B virus; Cases defined: ICD-9 or -10 = International Classification of Diseases, Ninth Revision or Tenth Revision, CM = Clinical Modification; Duration of follow-up: When the follow-up periods of statin user and nonuser were different, only the shorter one was showed, and all periods were transformed to years; Statin type: A = Atorvastatin, C = Cerivastatin, F = Fluvastatin, L = Lovastatin, P = Pravastatin, R = Rosuvastatin, S = Simvastatin, Non-statin = Non-statin cholesterol-lowering drug(s) only; Duration of statin use: M = Mean, Me = Median, ≥1 cDDD = more than 1 cumulative defined daily dose before the diagnosis of liver cancer, Rx = prescriptions.

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Table 2. Study data

Studies	Intervention/ Cases		Control		Measurements of effect estimates	Crude RR with 95% CIs	Adjusted RR with 95% CIs	Confounders for adjustment
	No. of event/ No. of exposure	No. of total	No. of event/ No. of exposure	No. of total				
Emberson, 2012, UK [19]	35	67258	33	67279	RR	1.06 (0.66, 1.71)*	1.06 (0.66, 1.71)*	Randomization
Friis, 2005, North Jutland [28]	1	12251	166	334754	OR	NA	1.16 (0.46-2.90)	1,2, 16, 21, 23
Friedman(Male), 2008, USA [29]	32	192598	NA	1904876	HR	NA	0.49 (0.34-0.70)	16
Friedman(Female), 2008, USA [29]	10	169261	NA	1976332	HR	NA	0.40 (0.21-0.75)	
Marelli, 2011, USA [30]	13	45857	24	45857	RR	0.31 (0.14-0.68)*	0.31 (0.14-0.68)*	1-5, 14, 16-18, 26, 27
Tsan, 2012, Taiwan [31]	58	2785	963	30628	HR	0.66 (0.51- 0.86)	0.47 (0.36-0.61)	1, 2, 7, 8, 11, 12
Tsan, 2013, Taiwan [32]	1378	35023	26505	225841	HR	0.42 (0.39-0.46)	0.53 (0.49–0.58)	1, 2, 7, 8, 11, 13
Khurana, 2005, USA [33]	NA	NA	NA	NA	OR	NA	0.52 (0.41- 0.67)	1, 11, 13
El-Serag, 2009, USA [34]	447	1303	2766	5212	OR	0.46 (0.40-0.52)	0.74 (0.64-0.87)	1-3, 6, 8, 9, 11-13, 21, 24, 28
Chiu, 2011, Taiwan [35]	117	1166	195	1166	OR	0.53 (0.41-0.69)	0.62 (0.45-0.83)	1, 2, 8, 9, 11, 12, 20, 29
Lai, 2013, Taiwan [36]	255	3480	1635	13920	OR	0.61 (0.52–0.72)	0.71 (0.56–0.89)	1, 2, 8-13, 22, 24, 25
Leung, 2013, Taiwan [37]	26	424	6851	33781	HR	0.45 (0.30-0.67)	0.44 (0.28, 0.72)	1, 2, 11, 15, 20, 21, 23
Chaiteerakij, 2013, USA [38]	72	165	165	256	OR	NA	0.6 (0.4-0.9)	1-3, 8, 11, 17, 22, 28, 30

The RR with an asterisk mark (*) was calculated based on the raw data. The others, crude or adjusted, were extracted from the original paper; Confounders for adjustment: 1 = age, 2 = sex, 3 = race, 4 = BMI, 5 = smoking status, 6 = ethanol intake, 7 = socioeconomic status, 8 = cirrhosis, 9 = alcoholic liver disease, 10 = non-alcoholic fatty liver disease, 11 = diabetes mellitus, 12 = HBV infection, 13 = HCV infection, 14 = concomitant diagnoses (unspecified), 15 = Charlson score, 16 = calendar year, 17 = cholesterol (totalcholesterol, VLDL, LDL, or triglycerides), 18 = prostate-specific antigen, 19 = resection extent, 20 = other lipid-lowering agents, 21 = cardiovascular medications (aspirin, nonsteroidal anti-inflammatory medications, or angiotensin-converting enzymes inhibitors), 22 = metformin or thiazolidinedione, 23 = hormone-replacement therapy, 24 = HCV treatment, 25 = HBV treatment, 26 = medications taken (unspecified), 27 = the number of office visits, 28 = propensity to use statins, 29 = hospital stay, 30 = biliary tract diseases

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Table 3. Subgroup analyses of included studies

Subgroup		No. of studies (reports)	Summary RR (95% CIs)	Heterogeneity, I^2	Heterogeneity, *P* value	$P_{interaction}$
Study design	RCT	1	1.06 (0.66-1.71)	-	-	***P = 0.01***
	Observational studies	11(12)	0.57(0.50-0.64)	61%	***P* = 0.003**	
Observational studies	Cohort studies	5 (6)	0.51 (0.44–0.58)	18%	*P* = 0.30	***P* = 0.04**
	Case-control studies	6	0.63 (0.54–0.73)	46%	***P* = 0.10**	
Baseline risk of liver cancer	Higher baseline risk	4	0.52 (0.47-0.59)	16%	*P* = 0.31	***P*** = 0.08
	General population	8 (9)	0.63 (0.52–0.75)	59%	***P* = 0.01**	
Confounding adjustment	Adequate adjustment	6	0.64(0.53-0.77)	81%	***P* = 0.0001**	***P*** = 0.08
	Inadequate adjustment	6 (7)	0.51 (0.43-0.60)	3%	*P* = 0.40	
Study location	Western studies	7 (8)	0.61 (0.48-0.76)	64%	***P* = 0.007**	*P* = 0.54
	Asian studies	5	0.56 (0.48-0.64)	51%	***P* = 0.09**	
Pharmacokinetic	Hipophilic statins	5 (6)	0.57 (0.50-0.65)	50%	***P* = 0.08**	***P*** = 0.86
	Hydrophilia statins	3	0.59(0.41–0.84)	50%	***P* = 0.13**	
Higher cumulative dosage of statin		6	0.53 (0.36-0.79)	90%	***P*<0.0001**	-

RR = relative risk; higher baseline risk of liver cancer: patients with older age, HBV or HCV infection. Adequate adjustment: RCT or studies which adjusted for at least 4 of 7 important confounders, such as HBV infection, HCV infection, cirrhosis, alcoholic liver disease, NAFLD, HBV treatment, or HCV treatment; Lipophilic statins: Atorvastatin, Fluvastatin, Lovastatin, or Simvastatin; Hydrophilia statins: Pravastatin or Rosuvastatin; Higher cumulative dosage of statin use: > 180cumulative defined daily dose or Duration of statin use > 0.5 years before the diagnosis of liver cancer.

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Statin use and risk of liver cancer: an update meta-analysis

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Running title: Meta-analysis: statin and liver cancer

Key words: *Statin; Liver cancer; Cancer Prevention; Meta-analysis.*

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Abstract

Objective: Statins are commonly prescribed cholesterol-lowering drugs. Preclinical studies suggest that statins may possess cancer preventive properties. The primary objective of this meta-analysis was to determine the association between the statin use and the risk of liver cancer.

Design: Meta-analysis.

Setting: International.

Participants: A comprehensive literature search of PubMed, BIOSIS Previews, Web of Science, EMBASE, EBSCO and Cochrane Library was conducted through March 2014. The effect estimate was reported as pooled relative risk (RR) with 95% confidence intervals (CIs), using the random-effects model.

Results: A total of 12 studies (one individual patient data analysis of 22 randomized controlled trials, 5 cohorts, and 6 case-controls) were qualified for this meta-analysis, involving 5,640,313 participants including 35,756 liver cancer cases. Our results indicated a significant risk reduction of liver cancer among all statin users (RR 0.58, 95%CIs 0.51–0.67). The difference of the study designs can partly explained the significant heterogeneity found in the overall analysis (I^2 = 65%, P = 0.0006). No evidence of publication bias was observed in this meta-analysis. Similar risk reductions were found in the subgroups analysis of Western and Asian countries, lipophilic and hydrophilia statins. There was a trend toward more risk reductions in subgroups with higher baseline risk, inadequate adjustment, and higher cumulative dosage of statin use.

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Conclusions: This meta-analysis suggests that the statin is associated with a significant risk reduction of liver cancer, when taken daily for cardiovascular event prevention. However, this preventive effect might be overestimated due to the exposure period, tthe indication and contraindication of statins, and other confounders. Statins might be considered as an adjuvant in the treatment of liver cancer.

Key words: *Statin; Liver cancer; Cancer Prevention; Meta-analysis.*

Strengths and limitations of this study

Statins are commonly prescribed as cholesterol-lowering drugs. In this comprehensive meta-analysis, we demonstrate that the statin use is associated with a significant risk reduction of liver cancer.

The difference of the study designs is the part reason that explained the significant heterogeneity found in the overall analysis.

However, this preventive effect might be overestimated due to the exposure period, tthe indication and contraindication of statins, and other confounders.

Statins might be considered as an adjuvant in the treatment of liver cancer.

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Introduction

Statins are inhibitors of 3-hydroxy-3-methylglutaryl coenzyme-A (HMG-CoA) reductase and they are widely used to reduce the plasma cholesterol level and the risk of cardiovascular events.[1] Although there is a concern over their possible carcinogenicity raised in rodent studies,[2] preclinical studies indicate that statins have anticancer properties *in vitro* and *in vivo*, through inhibiting angiogenesis, inducing apoptosis, and suppressing tumor growth and metastasis.[3-5]

However, higher concentrations of statins are typically required to induce these effects, raising questions concerning the therapeutic relevance of statins on cancer.[6] To date, clinical studies regarding the cancer incidence associated with statin administration have highlighted conflicting results. Moreover, a large number of meta-analyses have concluded that there was no association between statin use and risk of overall cancer,[7-10] or cancer of breast[11], stomach,[12] or pancreas.[13] There is only a modest protective effect of statins in prostate cancer[14] and colorectal cancer.[15]

In contrary, recent studies reported encouraging results for risk reduction of liver cancer among all statin users. Previous meta-analysis, conducted by Singh *et al*. by including 10 studies, found that statin users were less likely to develop hepatocellular carcinoma (HCC) than statin non-users.[16] However, Singh *et al*. included the ALERT, LIPS, and MEGA trials twice, by including three individual patient data (IPD) analysis of randomized controlled trials (RCTs).[17-19] Meanwhile, some factors of stratification were not considered in their analyses, such as dose and timing of exposure to statins, and the selection of controls and confounders, which might limit

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the evaluation of cancer risk.[20] Furthermore, the lipophilic statins are accompanied by an extensive first-pass effect at the hepatic level.[21] It is plausible that lipophilic statins may have a better liver cancer preventive qualities than the hydrophilic ones.[22]

Therefore, we performed this updated meta-analysis to assess the association between the statin use and the risk of liver cancer, involving the recently published studies and conducting more subgroup analyses based on the factors mentioned above. Our results demonstrated that statin use was associated with an over 40% risk reduction in liver cancer, which may have a significant translational potential in the clinical practice. However, there were some confounders might overestimate this preventive effect of statins.

MATERIALS AND METHODS

Literature Search strategy

This meta-analysis was conducted following the PRISMA guidelines.[23]

The systematic computerized search for eligible studies were performed on the database of PubMed, BIOSIS Previews, Web of Science, EMBASE, EBSCO, and Cochrane Library, covering all studies published from their inception to March 5, 2014. The following terms were searched with both the subjects (MeSH terms) and text-word search strategies: "(Statin OR HMG-CoA reductase inhibitors OR Atorvastatin OR Cerivastatin OR Fluvastatin OR Lovastatin OR Pravastatin OR Rosuvastatin OR Simvastatin) AND (Hepatocellular OR Hepatic OR Intrahepatic OR Interlobular OR Liver) AND (Carcinoma OR Sarcomas OR Angiosarcoma OR Cancer OR Neoplasm). Additionally, the relevant reviews and retrieved articles were searched

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manually for more eligible studies.

In study searching, only the original researches, published in form of peer review article or meeting abstract, were included. No language restrictions were imposed. However, the studies we included were all published in English.

Study selection

The inclusion criteria were: (1) randomized controlled trial (RCTs), cohort studies or case-control studies; (2) original studies that assessed the effect of statin use on the risk of liver cancer, compared with placebo or no treatment; (3) liver cancer cases were identified according to the International Classification of Diseases codes (ICD); and (4) studies with estimate of relative risk (risk ratio, RR) of liver cancer, or with data sufficient to calculate it.

The exclusion criteria were: (1) study design not meeting the inclusion criteria; (2) studies without estimate of RR, or without sufficient data to calculate it; or (3) studies with duplicated or overlap reports.

Data extraction

Two independent investigators (M. Shi and X.B. Cui) extracted data from the eligible studies using a predefined data collection form. The differences of data extraction were resolved by consensus referring back to the original article. The extracted information included: (1) Studies: first author, year of publication, study design, location, patient populations, period, and follow-up; (2) Statins: type, dosage or duration of statin use; (3) liver cancer: case identification, number of liver cancer, crude RR with 95% confidence intervals (CIs), adjusted RR reflecting the greatest

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degree of control for confounders, and confounders for adjustment (including variables for matching). When the RR were not available, the RR with 95% CIs were calculated from the raw data in original studies.

We extracted different measurements of effect estimates from original studies, such as Relative Risk (RR), Odds Ratio (OR), Hazard Ratio (HR), and Observed/Expected ratio. Due to the fact that the incidence of liver cancer was low in all studies, theses different measurements can be used to provide similar estimates of RR.

Methodological quality assessment

Of note, the included RCT was pooled analysis of other RCTs, therefore, it is inappropriate to assess the methodological quality. The methodological quality of cohort and case-control studies were assessed on the Newcastle-Ottawa Scale,[24] including eight items that were categorized three categories: selection (four items, one star each), comparability (one item, up to two stars), and exposure/outcome (three items, one star each). A "star" presents a "high" quality choice of each item.

Statistical analysis

The overall meta-analysis was first performed, followed by the subgroup analyses, based on study design, baseline risk of liver cancer, confounding adjustment, study location, and pharmacokinetic. Meanwhile, we conducted subgroup analyses based on studies which reported RR estimate for higher cumulative dosage of statin use, when appropriate data were available.

To take into account the heterogeneity and provide a more conservative estimate, the inverse variance method was used to estimate the pooled RR and corresponding 95%

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CIs, and data were pooled using a random effects model. Heterogeneity was assessed using the Chi-squared statistic (P) together with the Higgins I-squared statistic (I^2), a P value <0.10 was considered statistically significant for heterogeneity; and an I^2 value > 50 % was considered a measure of severe heterogeneity.[25]

Publication bias was assessed using the Begg's test and the Egger's test.[26] Influence analysis was performed to investigate the influence of a single study on the overall meta-analysis estimate, by omitting one study in each turn. Test for interaction was applied to identify the difference between pooled RR from subgroup analysis using the method described by Altman and Bland.[27] All statistical tests were two-sided and $P < 0.05$ was considered statistically significant, unless otherwise specified. Software Review Manager (RevMan5.2, Copenhagen) and STATA (Stata 11.2, Texas) were used for the statistical analysis.

Results

Study selection

Figure 1 illustrated the process of study selection for the meta-analysis. Of the 1424 potentially relevant references identified by electric and manual search, 142 were selected for full-text review after screening titles and abstracts. Finally, a total of 12 studies were included, with one IPD analysis,[19] five cohort studies,[28-32] and six case-control studies.[33-38] One case-control study was presented solely in abstract form.[33]

Of note, the cohort study conducted by Friedman *et al.* reported RR estimate separately for different gender (male and female),[29] we considered these two reports

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as separate studies. Therefore, a total of thirteen reports were included for the present meta-analysis.

Study characteristics

Table 1 summarized the characteristics of qualified studies in this meta-analysis. The 12 studies, involving 5,640,313 participants with 35,756 liver cancer cases, were published between 2005 and 2013. The "RCT" in the present study was pooled analysis of 22 clinical trials,[19] which investigated statins therapy in cardiovascular event prevention and reported the occurrence of liver cancer as adverse event. The observational studies were conducted with the local or national health databases, the statin exposure were identified by linkage to prescription databases, and the controls were matched mainly by age, sex and index date. Except one cohort adopted ICD-10 C22,[28] all other studies identified liver cancer cases according to the ICD-9 155. Of note, two cohorts were restricted to patients with HBV infection,[31] and HCV infection;[32] one case-control only included patients with diabetes mellitus;[34] two observational studies included patients aged at least 45 years.[30 35]

Table 2 summarized the data of the included studies. In the RCT[19] and one cohort study,[30] the RR with 95% CIs were calculated from the 2×2 tables defined by the incidence of liver cancer and the statin use status. The observational studies reported different measurements of RR estimates with adjustment by confounders. Several observational studies adopted the important risk factors of liver cancer for adjustments[31 32 34-36], such as HBV infection, HCV infection, cirrhosis, alcoholic liver disease, or non-alcoholic fatty liver disease (NAFLD).[39] Of note, only two studies

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adjusted for the cholesterol level,[30 38] and no study adjusted for the metabolic syndrome, which might also influence the risk of liver cancer.[39]

Methodological quality

For the cohort and case-control studies, the median score was 7 on the Newcastle-Ottawa Scale, with a range of 5 to 8 (**Supplementary Table 1**). These results indicated that the observational studies were in a reasonable good quality.

Overall meta-analysis

Figure 2 depicted the forest plot of RR estimate with 95% CIs from individual studies and overall meta-analysis. In the overall meta-analysis, pooled results showed a statistically significant decrease in the liver cancer risk among all statin users (RR 0.58, 95%CIs 0.51–0.67). Of note, a statistically significant heterogeneity was observed (I^2 = 65%, P = 0.0006). The P-values of Begg's test and Egger's test were 0.669 and 0.749, respectively, both suggesting there was no evidence of publication bias. In the influence analysis, the omission of any individual studies did not alter the direction and magnitude of the observed effect (**Supplementary Figure 1**).

Subgroup analyses and Test for interaction

We first performed preplanned subgroup analyses based on study design, baseline risk of liver cancer, confounding adjustment, and study location (**Table 3**).

The RCT showed there is no significant association between statin use and risk of liver cancer (RR 1.06, 0.66–1.71). But the observational studies indicated a significant decrease of liver cancer risk among all statin users (RR 0.57, 0.50–0.64; I^2 = 61%, P = 0.003) (**Figure 2**). Furthermore, we found a greater risk reduction in the subgroup

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analysis of cohort studies (RR 0.51, 0.44–0.58; I^2 = 18%, P = 0.30) than in the case-control studies (RR 0.63, 0.54–0.73; I^2 = 46%, P = 0.10) (**Supplementary Figure 2**).

Test for interaction showed significant results between subgroups of the RCT and observational studies ($P_{interaction}$ = 0.01, Z = 2.47), and between subgroups of the cohort and case-control studies ($P_{interaction}$ = 0.04, Z = -2.03). These results indicated that the difference of the study designs was the part reason that why there was severe heterogeneity in the overall analysis (**Table 3**).

In the subgroup analysis of the four studies with higher baseline risk of liver cancer,[30-32 35] defined as patients with older age, HBV or HCV infection, there was a trend toward more decrease of liver cancer risk (RR 0.52, 0.47-0.59; I^2 = 16%, P = 0.31) than in the other eight studies with general population[19 28 29 33 34 36-38] (RR 0.63, 0.52–0.75; I^2 = 59%, P = 0.01) (**Supplementary Figure 3**).

We defined the RCT or studies adjusted for at least 4 of 7 important confounders, such as HBV infection, HCV infection, cirrhosis, alcoholic liver disease, NAFLD, HBV treatment, or HCV treatment,[39] were adjusted adequately. Subgroup analysis of these six studies[19 31 32 34-36] found a trend toward less decrease of liver cancer risk (RR 0.64, 0.53-0.77; I^2 = 81% P = 0.0001) than the other six studies[28-30 33 37 38] (RR, 0.51, 0.43-0.60; I^2 = 3%, P = 0.40) (**Supplementary Figure 4**).

Subgroup analyses based on study location found a similar risk reduction of liver cancer in the Western countries (RR 0.61, 0.48–0.76; I^2 = 64%, P = 0.007) and in the Asian countries (RR 0.56, 0.48–0.64; I^2 = 51%, P = 0.09). (**Supplementary Figure 5**)

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Besides the overall RR estimates, some studies reported different RR estimate for different pharmacokinetic and dosage of statin use (**Supplementary Table 2)**. We conducted further subgroup analyses based on these available data.

According to the different pharmacokinetic, statins can be classified as lipophilic statins (Atorvastatin, Fluvastatin, Lovastatin, and Simvastatin) and hydrophilia statins (Pravastatin and Rosuvastatin).[21] Subgroup analysis of lipophilic statins [29 31 34-36] found a significant decrease of liver cancer risk (RR 0.57, 0.50–0.65; I^2 = 50%, P = 0.08). And there was a similar result among users of hydrophilia statins[31 35 36] (RR 0.59, 0.41–0.84; I^2 = 50%, P = 0.13) (**Supplementary Figure 6**).

Test for interaction showed non-significant results for subgroups with different baseline risk, confounding adjustment, study location, or pharmacokinetic ($P_{interaction}$ = 0.08, 0.08, 0.54 and 0.86, respectively) (**Table 3**). Therefore, there is no strong evidence to support a different preventive effect of statins on liver cancer in these subgroups.

Subgroup analysis of six studies with higher cumulative dose of statin use, defined as statin use more than 180 cumulative defined daily dose (cDDDs) or 0.5 years (cumulative duration), showed a trend toward more risk reduction of liver cancer (RR 0.53, 0.36-0.79), but with a high degree of heterogeneity (I^2 = 90%, P<0.00001) (**Supplementary Figure 7**).

Discussion

This present meta-analysis represents the most comprehensive review to date on the association between the statin use and the liver cancer risk, by including 12 studies

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(one IPD analysis of 22 RCTs, 5 cohort studies, and 6 case-control studies) and involving 5,640,313 participants with 35,756 liver cancer cases. Overall, we found that statin use was associated with an over 40% risk reduction in liver cancer compared with nonusers (RR 0.58, 95%CIs0.51–0.67). This result was in line with the previous three meta-analyses: Singh *et al.* included 10 studies and suggested statin users were less likely to develop HCC (OR 0.63, 95%CIs 0.52-0.76),[16] Pradelli *et al.* and Zhang *et al.* included 5 and 7 observational studies and found a summary RR of 0.58 (95%CIs 0.46–0.74) and 0.61 (95%CIs 0.49–0.76), respectively.[40 41]

The IPD analysis of 22 RCTs showed there is no significant association between statin use and risk of liver cancer. The significant risk reduction of liver cancer among all statin users was seen primarily in the observational studies, and this preventive effect was relatively convinced in the cohorts than in the case-controls. There were some reasons to explain the different findings between RCTs and observational studies.

First, the exposure period to statins might be shorter than the period to carcinogenesis and the latency to diagnosis in the cohorts and the case-controls. The observational studies defined statin use varying in dosage and duration, from patients who received ≥1 cDDD or >1 Rx of statins to more than 0.5 years (**Table1**). On the other hand, the median period of statin use was 5.1 years in the RCTs. Although there was a trend toward more risk reduction of liver cancer with higher cumulative dose of statin use, this defect might still result in overestimating the cancer-preventive effect of statins in the observational studies.

Second, clinical studies demonstrated that higher serum total cholesterol

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concentration was associated with decreased risk of liver cancer (**Supplementary Table 3**).[42-44] Meanwhile, there were inverse association between use of non-statin lipid-lowering drugs and risk of the liver cancer.[35 38] Meanwhile, because of the contraindication, statins might not prescribed to the patients with the chronic liver disease, which is known as a risk factor of liver cancer. Unfortunately, the observational studies included in this analysis seldom adopted these factors for adjustment. Actually, subgroup analysis of studies with adequate adjustment showed a trend toward less risk reduction, indicating the potential of overestimate this preventive effect by confounders.

Third, the RCTs included lower risk population (patients with cardiovascular disease rather than HBV /HCV infection), might not be powerful enough to investigate the liver cancer outcomes, which were much rarer than cardiovascular events. In addition, subgroup analysis of studies with higher baseline risk showed a trend toward more decrease of liver cancer risk.

These reasons suggested that the observed modulation of cancer incidence cannot be ascribable to a direct statin-mediated effect,[20] the exposure period, the indication (e.g. hyperlipidemia) and contraindication (e.g. chronic liver disease) of statins might overestimate its cancer-preventive effect.

We found similar results in Western countries and Asian countries, which were different from the meta-analysis conducted by Singh *et al.* which concluded that the inverse association of statins with HCC was stronger in the Asian population. Considering four more studies we included, this difference might be caused by the

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insufficient data in their meta-analysis. Based on the pharmacokinetics, it is plausible that lipophilic and hydrophilic statins will differ in their liver cancer prevention qualities.[21 22] However, subgroup analysis of lipophilic and hydrophilic statins showed similar results.

Besides the limitations described previously, there were some other limitations should be noted. First, a significant heterogeneity was observed in the present meta-analysis, which might results from the difference in study design. Results of subgroup analyses would also be limited by this heterogeneity. Second, the adherence to statin therapy is known to be associated with healthy lifestyle, which might affect the cancer outcome.[45] Such information is hard to be captured in databases or medical record in the observational studies.[46] Third, five observational studies were conducted using the Taiwanese National Health Insurance Research Database (NHIRD),[31 32 35-37] although they were not in the same period, these studies might contain overlapping groups of patients. These limitations mentioned above might lead to confounding of overall results from the present study, and should be considered in future studies aiming at confirming the protective effects of statins on human cancer risk.

The strengths of our meta-analysis were as follows: First, we performed a much more comprehensive search and more subgroup analyses, compared with the previous meta-analyses; Second, the methodological quality of the included studies were reasonable good; Third, publication bias, which due to the tendency of not publishing small studies with null results, were not found in our meta-analysis.

Of note, preclinical studies have indicated that statins possess synergism with other

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therapeutic agents *in vitro* and *in vivo* for liver cancer.[47 48] Some clinical studies have also demonstrated that statins would prolong survival in patients with advanced liver cancer (**Supplementary Table 4**),[49-52] and associated with risk reduction of recurrence after curative surgery in patients of HBV related HCC.[53] Therefore, considerable interest exists in adjunctive therapy with statins for liver cancer. In fact, there were some RCTs ongoing to determine the effectiveness of pravastatin, when used in combination with sorafenib, in the treatment of liver cancer (**Supplementary Table 5**).

Currently, physicians are less likely to prescribe statins for patients with chronic liver disease, based on the concerns about the statin-induced liver injury.[31] However, there were number of studies have demonstrated the safe use, even salutary effects.[54-56] Meanwhile, the risk of serious statin-related liver injury appears to be no greater than the background incidence of this rare event.[57] Therefore, considering their benefits for cardiovascular event prevention and the potential effect in liver cancer prevention and treatment, statins should not be denied to these patients.

In conclusion, our results suggest that statin use is associated with a significant risk reduction of liver cancer, when taken daily for cardiovascular event prevention. However, this preventive effect might be overestimated due to the exposure period, indication and contraindication of statins, and other confounders. Statins might be considered as an adjuvant in the treatment of liver cancer.

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CONTRIBUTORSHIP STATEMENT

XB Cui had the original idea, M Shi, XB Cui and W Gong worked together to develop an appropriate theoretical framework and design. XB Cui developed the search, M Shi and XB Cui were involved in the selection process. M Shi and XB Cui extracted relevant data, XB Cui and W Gong performed the statistical analysis and all authors were involved in the data interpretation. M Shi and B Nie wrote the manuscript draft and revised the draft based on input from the other authors. All authors revised it critically for content and approved the final version.

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There are no competing interests

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No additional data available.

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Figure legends:

Figure 1. Flow chart of study selection in the present meta-analysis.

Figure 2. Overall meta-analysis of the statin use and the liver cancer risk.

Supplementary Figure 1. Influence analysis.

Supplementary Figure 2. Subgroup analyses based on study design.

Supplementary Figure 3. Subgroup analyses based on baseline risk of liver cancer.

Supplementary Figure 4. Subgroup analyses based on confounder adjustment.

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Supplementary Figure 5. Subgroup analyses based on study location.

Supplementary Figure 6. Subgroup analyses based on pharmacokinetic of statins.

Supplementary Figure 7. Subgroup analysis of higher cumulative dose of statin use.

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Table 1. Study characteristics

Studies	Study design	Patient population	Study period	Cases defined	Follow-up	Statins type	Dosage/Duration of Statin use
Emberson, 2012, UK [19]	RCT	IPD analysis of 22 RCTs	-	ICD-9 155	5.1 years (Me)	A, F, L, P, R, S	5.1 years (Me)
Friis, 2005, North Jutland [28]	Cohort	General population (CPR)	1989-2002	ICD-10 C22	3.3 years (M)	Unspecified	≥2 Rx
Friedman, 2008, USA [29]	Cohort	General population (KPMCP)	1994-2003	ICD-9-CM 155	> 2 years	A, L, S (97.6%)	≥1 Rx
Marelli, 2011, USA [30]	Cohort	General older population (men ≥ 45 and women ≥ 55 years; GE Centricity)	1990-2009	ICD-9 155	4.6 years (M)	Unspecified	≥1 cDDD
Tsan, 2012, Taiwan [31]	Cohort	Patients with HBV infection (NHIRD)	1997-2008	ICD-9 155	9.9 years (M)	A, F, L, P, R, and S	≥28 cDDDs
Tsan, 2013, Taiwan [32]	Cohort	Patients with HCV infection (NHIRD)	1999-2010	ICD-9 155	10.7 years (M)	A, F, L, P, R, and S	≥28 cDDDs
Khurana, 2005, USA [33]	Case control	General population (VISN)	1997-2002	ICD-9 155	NR	Unspecified	≥1 Rx
El-Serag, 2009, USA [34]	Case control	Diabetes patients (VA)	1997-2002	ICD-9-CM 155	2.4 years (M)	A, C, F, L, P, and S	1.6 years (M)
Chiu, 2011, Taiwan [35]	Case control	Older patients(≥ 50 years; NHIRD)	2005–2008	ICD-9-CM 155	NR	A, F, L, P, R, and S	≥ 1 cDDD
Lai, 2013, Taiwan [36]	Case control	General population (NHIRD)	2000-2009	ICD-9-CM 155	1.4 years (M)	A, F, L, P, R, and S	≥1 Rx
Leung, 2013, Taiwan [37]	Case control	General population (NHIRD)	2000-2008	ICD-9-CM 155	4.1 years (M)	Unspecified	> 0.5 years
Chaiteerakij, 2013, USA [38]	Case control	Hyperlipidemia patients (Mayo Clinic)	2000-2010	ICD-9-CM 155	>1 years	Unspecified	≥1 Rx

Patients population: IPD = Individual patient data, RCT = randomized controlled trials, CRP = the Central Population Register of Danish citizens, KPMCP = the Kaiser Permanente Medical Care Program in northern California, GE Centricity = the General Electric Centricity database, NHIRD = the Taiwanese National Health Insurance research database, VISN = Veterans Integrated Service Networks 16 Veteran Affairs database, VA = Veterans Affairs national databases, Mayo Clinic = Mayo Clinic (Rochester, MN), HBV = hepatitis B virus; Cases defined: ICD-9 or -10 = International Classification of Diseases, Ninth Revision or Tenth Revision, CM = Clinical Modification; Duration of follow-up: When the follow-up periods of statin user and nonuser were different, only the shorter one was showed, and all periods were transformed to years; Statin type: A = Atorvastatin, C = Cerivastatin, F = Fluvastatin, L = Lovastatin, P = Pravastatin, R = Rosuvastatin, S = Simvastatin, Non-statin = Non-statin cholesterol-lowering drug(s) only; Duration of statin use: M = Mean, Me = Median, ≥1 cDDD = more than 1 cumulative defined daily dose before the diagnosis of liver cancer, Rx = prescriptions.

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Table 2. Study data

Studies	Intervention/ Cases		Control		Measurements of effect estimates	Crude RR with 95% CIs	Adjusted RR with 95% CIs	Confounders for adjustment
	No. of event/ No. of exposure	No. of total	No. of event/ No. of exposure	No. of total				
Emberson, 2012, UK [19]	35	67258	33	67279	RR	1.06 (0.66, 1.71)*	1.06 (0.66, 1.71)*	Randomization
Friis, 2005, North Jutland [28]	1	12251	166	334754	OR	NA	1.16 (0.46-2.90)	1,2, 16, 21, 23
Friedman(Male), 2008, USA [29]	32	192598	NA	1904876	HR	NA	0.49 (0.34-0.70)	16
Friedman(Female), 2008, USA [29]	10	169261	NA	1976332	HR	NA	0.40 (0.21-0.75)	
Marelli, 2011, USA [30]	13	45857	24	45857	RR	0.31 (0.14-0.68)*	0.31 (0.14-0.68)*	1-5, 14, 16-18, 26, 27
Tsan, 2012, Taiwan [31]	58	2785	963	30628	HR	0.66 (0.51- 0.86)	0.47 (0.36-0.61)	1, 2, 7, 8, 11, 12
Tsan, 2013, Taiwan [32]	1378	35023	26505	225841	HR	0.42 (0.39-0.46)	0.53 (0.49–0.58)	1, 2, 7, 8, 11, 13
Khurana, 2005, USA [33]	NA	NA	NA	NA	OR	NA	0.52 (0.41- 0.67)	1, 11, 13
El-Serag, 2009, USA [34]	447	1303	2766	5212	OR	0.46 (0.40-0.52)	0.74 (0.64-0.87)	1-3, 6, 8, 9, 11-13, 21, 24, 28
Chiu, 2011, Taiwan [35]	117	1166	195	1166	OR	0.53 (0.41-0.69)	0.62 (0.45-0.83)	1, 2, 8, 9, 11, 12, 20, 29
Lai, 2013, Taiwan [36]	255	3480	1635	13920	OR	0.61 (0.52–0.72)	0.71 (0.56–0.89)	1, 2, 8-13, 22, 24, 25
Leung, 2013, Taiwan [37]	26	424	6851	33781	HR	0.45 (0.30-0.67)	0.44 (0.28, 0.72)	1, 2, 11, 15, 20, 21, 23
Chaiteerakij, 2013, USA [38]	72	165	165	256	OR	NA	0.6 (0.4-0.9)	1-3, 8, 11, 17, 22, 28, 30

The RR with an asterisk mark (*) was calculated based on the raw data. The others, crude or adjusted, were extracted from the original paper; Confounders for adjustment: 1 = age, 2 = sex, 3 = race, 4 = BMI, 5 = smoking status, 6 = ethanol intake, 7 = socioeconomic status, 8 = cirrhosis, 9 = alcoholic liver disease, 10 = non-alcoholic fatty liver disease, 11 = diabetes mellitus, 12 = HBV infection, 13 = HCV infection, 14 = concomitant diagnoses (unspecified), 15 = Charlson score, 16 = calendar year, 17 = cholesterol (totalcholesterol, VLDL, LDL, or triglycerides), 18 = prostate-specific antigen, 19 = resection extent, 20 = other lipid-lowering agents, 21 = cardiovascular medications (aspirin, nonsteroidal anti-inflammatory medications, or angiotensin-converting enzymes inhibitors), 22 = metformin or thiazolidinedione, 23 = hormone-replacement therapy, 24 = HCV treatment, 25 = HBV treatment, 26 = medications taken (unspecified), 27 = the number of office visits, 28 = propensity to use statins, 29 = hospital stay, 30 = biliary tract diseases

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Table 3. Subgroup analyses of included studies

Subgroup		No. of studies (reports)	Summary RR (95% CIs)	Heterogeneity, I^2	Heterogeneity, *P* value	$P_{interaction}$
Study design	RCT	1	1.06 (0.66-1.71)	-	-	***P = 0.01***
	Observational studies	11(12)	0.57(0.50-0.64)	61%	***P* = 0.003**	
Observational studies	Cohort studies	5 (6)	0.51 (0.44–0.58)	18%	*P* = 0.30	***P* = 0.04**
	Case-control studies	6	0.63 (0.54–0.73)	46%	***P* = 0.10**	
Baseline risk of liver cancer	Higher baseline risk	4	0.52 (0.47-0.59)	16%	*P* = 0.31	***P*** = 0.08
	General population	8 (9)	0.63 (0.52–0.75)	59%	***P* = 0.01**	
Confounding adjustment	Adequate adjustment	6	0.64(0.53-0.77)	81%	***P* = 0.0001**	***P*** = 0.08
	Inadequate adjustment	6 (7)	0.51 (0.43-0.60)	3%	*P* = 0.40	
Study location	Western studies	7 (8)	0.61 (0.48-0.76)	64%	***P* = 0.007**	*P* = 0.54
	Asian studies	5	0.56 (0.48-0.64)	51%	***P* = 0.09**	
Pharmacokinetic	Hipophilic statins	5 (6)	0.57 (0.50-0.65)	50%	***P* = 0.08**	***P*** = 0.86
	Hydrophilia statins	3	0.59(0.41–0.84)	50%	***P* = 0.13**	
Higher cumulative dosage of statin		6	0.53 (0.36-0.79)	90%	***P*<0.0001**	-

RR = relative risk; higher baseline risk of liver cancer: patients with older age, HBV or HCV infection. Adequate adjustment: RCT or studies which adjusted for at least 4 of 7 important confounders, such as HBV infection, HCV infection, cirrhosis, alcoholic liver disease, NAFLD, HBV treatment, or HCV treatment; Lipophilic statins: Atorvastatin, Fluvastatin, Lovastatin, or Simvastatin; Hydrophilia statins: Pravastatin or Rosuvastatin; Higher cumulative dosage of statin use: > 180cumulative defined daily dose or Duration of statin use > 0.5 years before the diagnosis of liver cancer.

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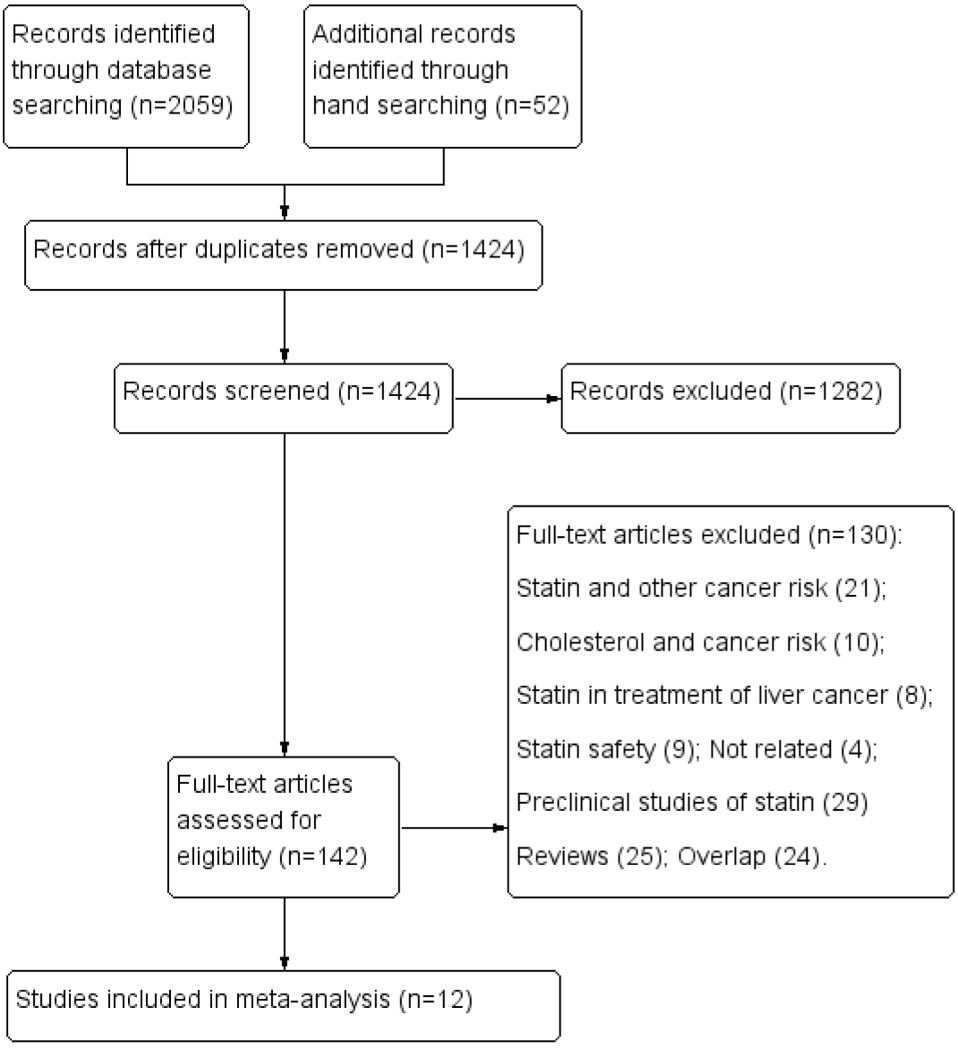

Flow chart of study selection in the present meta-analysis.
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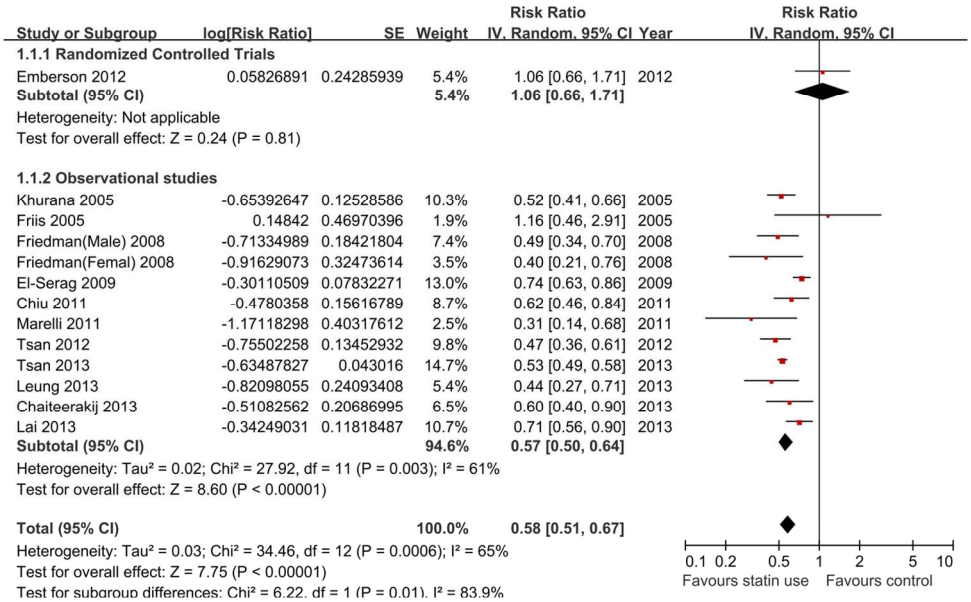

Overall meta-analysis of the statin use and the liver cancer risk.
128x80mm (300 x 300 DPI)

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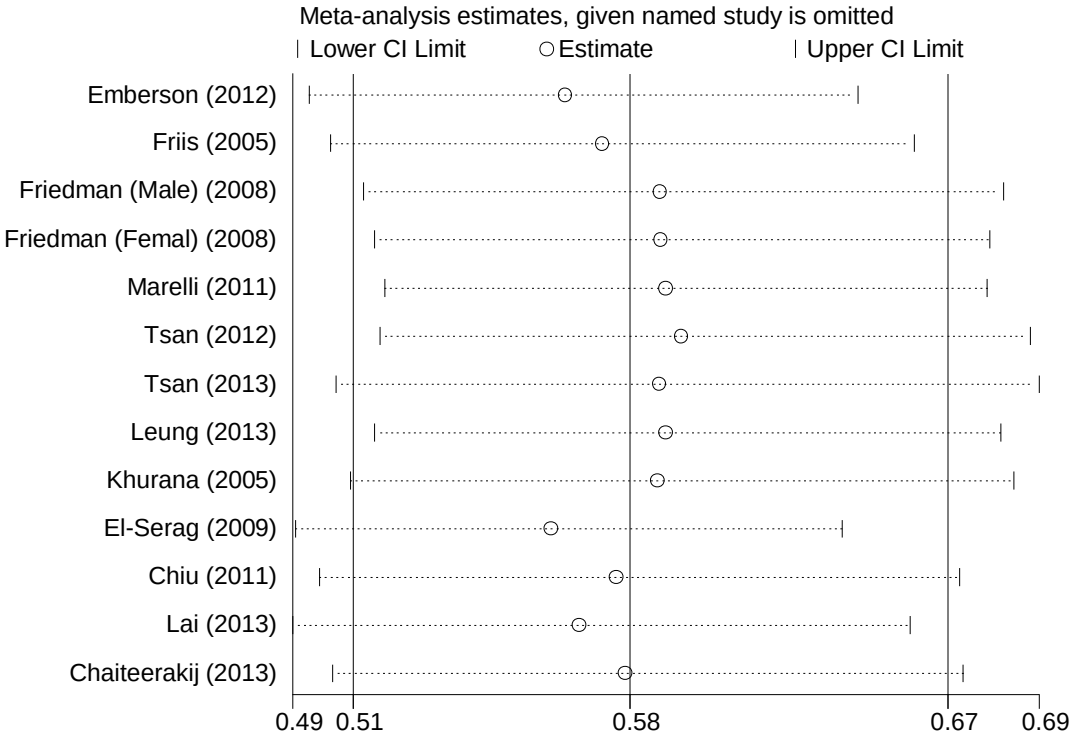
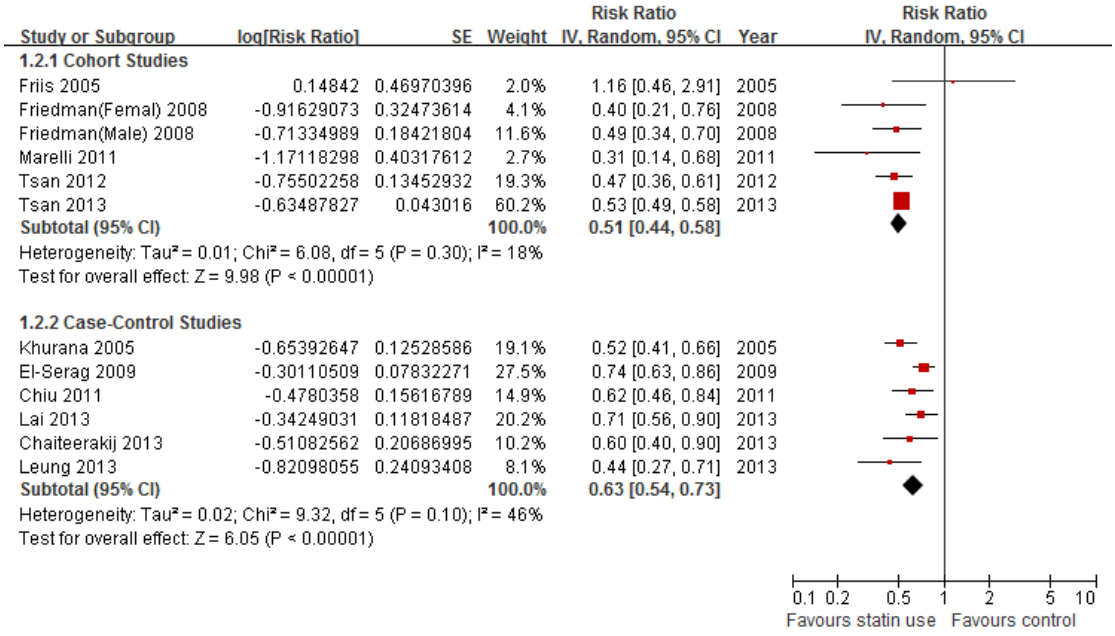
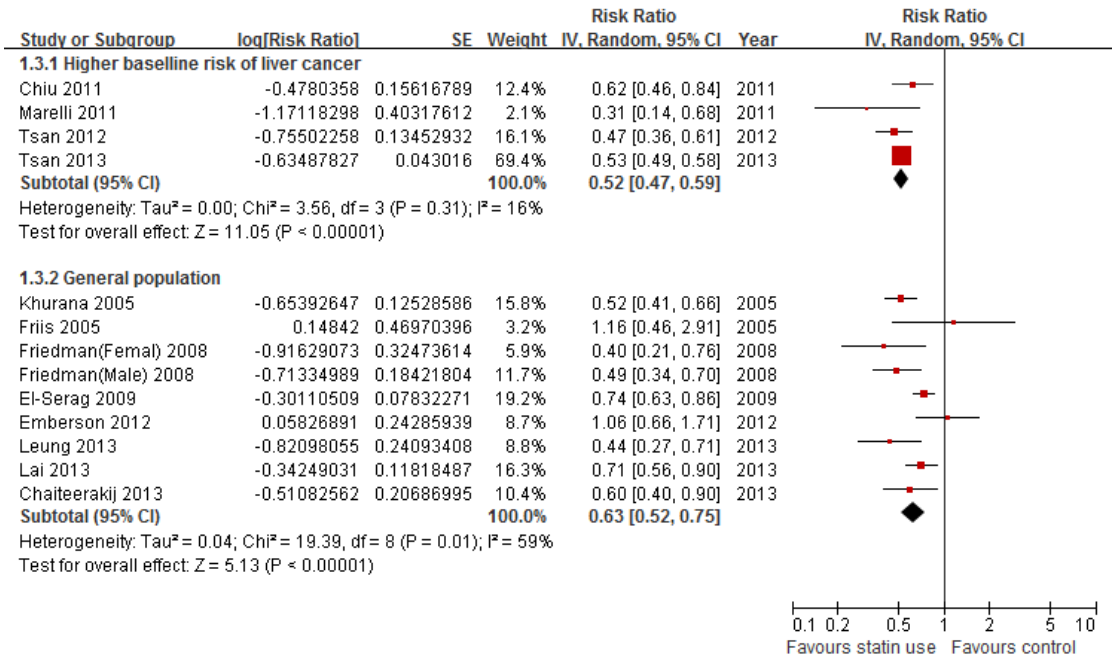
SUPPLEMENTARY FIGURES:



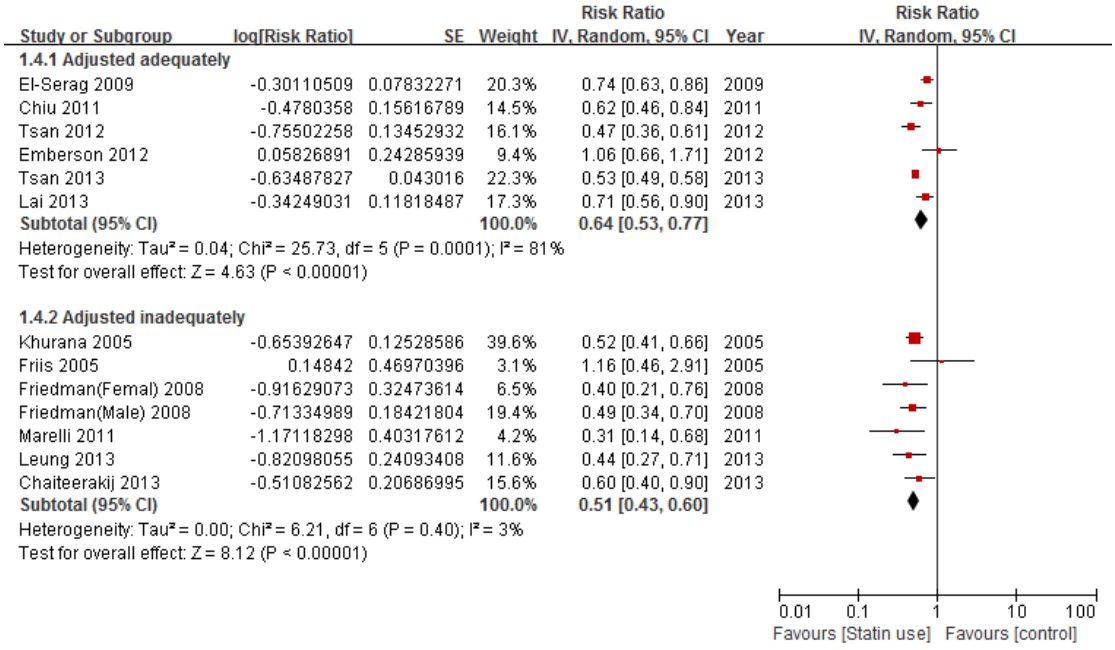
Supplementary Figure 1. Influence analysis.



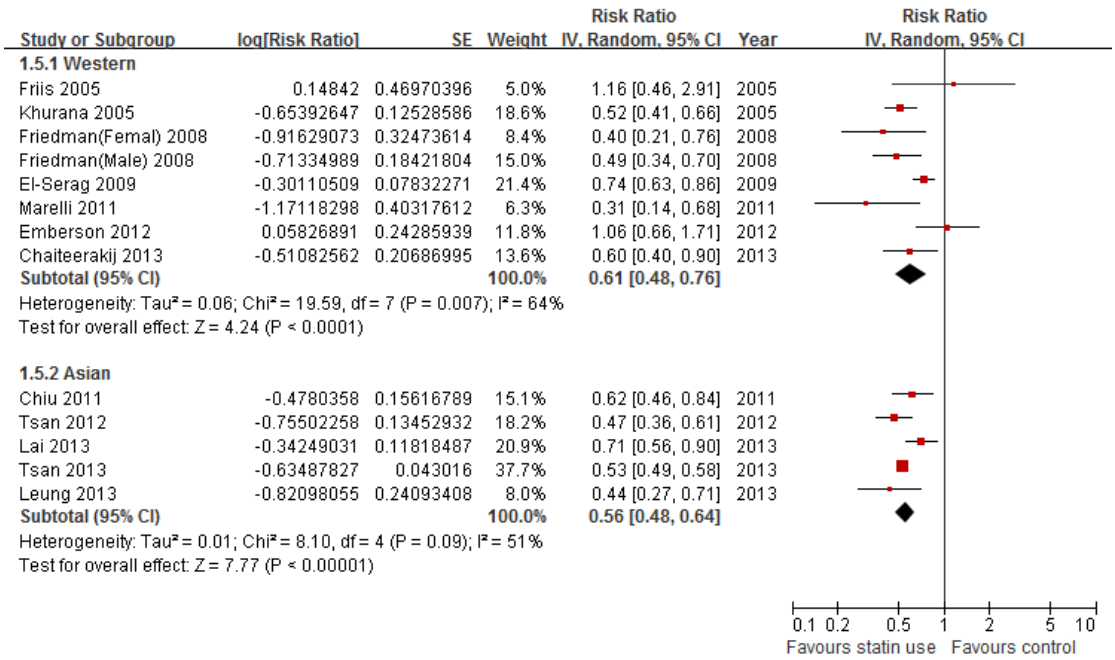
Supplementary Figure 2. Subgroup analyses based on study design.



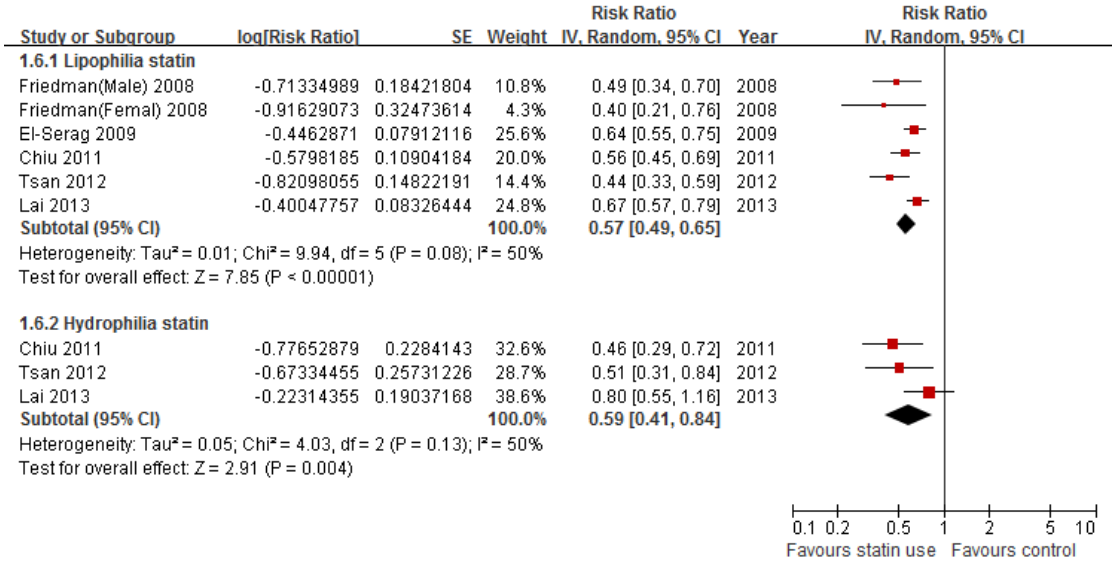
Supplementary Figure 3. Subgroup analyses based on baseline risk of liver cancer.



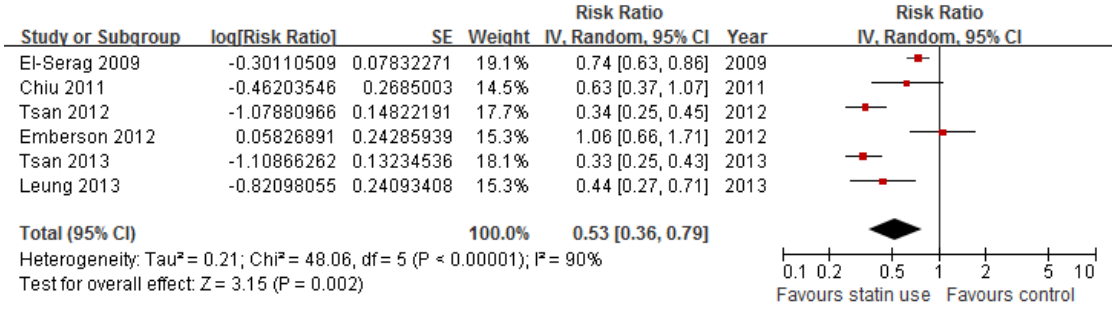
Supplementary Figure 4. Subgroup analyses based on confounder adjustment.



Supplementary Figure 5. Subgroup analyses based on study location.



Supplementary Figure 6. Subgroup analyses based on pharmacokinetic of statins.



Supplementary Figure 7. Subgroup analysis of higher cumulative dose of statin use.

SUPPLEMENTARY TABLES:

Supplementary Table 1. Assessment of methodological quality of the cohort and case-control studies according to the Newcastle–Ottawa Scale

Cohort Studies	**Selection**				**Comparability**	**Outcome**			**Total Score**
	Representativeness of the exposed cohort	Selection of the non-exposed cohort	Ascertainment of exposure	Outcome of present at start of study	Control for important factor	Assessment of outcome	Follow-up long enough	Adequacy of follow up	
Friis, 2005 [28]	☆	☆	☆	☆	☆	☆	-	☆	7
Friedman, 2008 [29]	☆	☆	☆	☆	☆	☆	-	☆	7
Marelli, 2011 [30]	☆	☆	☆	☆	☆	☆	☆	☆	8
Tsan, 2012 [31]	☆	☆	☆	☆	☆	☆	☆	☆	8
Tsan, 2013 [32]	☆	☆	☆	☆	☆	☆	☆	☆	8
Case–Control Studies	**Selection**				**Comparability**	**Exposure**			**Total Score**
	Adequate definition of cases	Representativeness of cases	Selection of controls	Definition of controls	Control for important factor	Ascertainment of Exposure	Same method for cases and controls	Non-response rate	
Khurana, 2005 [33]	-	☆	☆	☆	☆	☆	☆	-	6
El-Serag, 2009 [34]	-	☆	☆	☆	☆☆	☆	☆	-	7
Chiu, 2011 [35]	-	☆	☆	☆	☆☆	☆	☆	-	7
Lai, 2013 [36]	-	☆	☆	☆	☆☆	☆	☆	-	7
Leung, 2013 [37]	☆	☆	☆	☆	☆	☆	☆	☆	8
Chaiteerakij, 2013 [38]	-	☆	-	☆	☆	☆	☆	-	5

Control for important factor: ☆Reported relative risk have been adjusted for at least 4 of 7 important factors: HBV infection, HCV infection, cirrhosis, NAFLD, HCV treatment, HBV treatment, anti-diabetic medications; ☆ Study controls for any additional factor. Assessment of outcome: ☆ record linkage. Follow-up long enough: ☆ follow up period ≥ 4 years. Adequate definition of cases: ☆ The case is defined with independent validation. Non-response rate: ☆ Same rate for both groups.

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Supplementary Table 2. Studies reporting RR for use of lipophilic or hydrophilia statins, and for higher cumulative dosage of statin use

Studies	Measurements of effect estimates	Statins type	Dosage/Duration of Statin use	Crude RR with 95% CIs	Adjusted RR with 95% CIs
Tsan, 2012, Taiwan [31]	HR	A, F, L, P, R, and S	>365 cDDDs	0.50 (0.26-0.96)	0.34 (0.33-0.59)
	HR	Lipophilia statin	≥28 cDDDs	0.62 (0.47-0.83)	0.44 (0.33-0.59)
	HR	Hydrophilia statin	≥28 cDDDs	0.65 (0.39 -1.09)	0.51 (0.31-0.85)
Tsan, 2013, Taiwan [32]	HR	A, F, L, P, R, and S	>180 cDDDs	NA	0.33 (0.25–0.42)
El-Serag, 2009, USA [34]	OR	Simvastatin	1.6 years (M)	0.47 (0.41- 0.54)	0.64 (0.55-0.75)
Chiu, 2011, Taiwan [35]	OR	A, F, L, P, R, and S	>215.4 cDDDs	0.47 (0.30-0.72)	0.63 (0.37-1.06)
	OR	Lipophilia statin	≥ 1 cDDD	NA	0.56 (0.45–0.69)*
	OR	Hydrophilia statin	≥ 1 cDDD	NA	0.46 (0.29–0.71)*
Lai, 2013, Taiwan [36]	OR	Lipophilia statin	≥1 Rx	0.54 (0.48–0.61)*	0.67 (0.57–0.79)*
	OR	Hydrophilia statin	≥1 Rx	0.63 (0.47–0.83)*	0.80 (0.55–1.16)*

The RR with an asterisk mark (*) was calculated based on the raw data in the original study. The others, crude or adjusted, were extracted from the original paper.

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Supplementary Table 3. Published studies of the total cholesteroland the risk of liver cancer

Studies	Study design	cases/ participants	Follow-up	Reference (mg/dL)	Index (mg/dL)	Adjusted HR (95% CIs)		*P* for trend*	Confounders for adjustment
						Men	Women		
Iso, 2009, Japan [43]	Population-based cohort (JPHC Study)	125 /33,368	12.4 years	180–199	<160	2.62 (1.44–4.76)	4.15 (1.70–10.16)	Men < 0.0001 Women < 0.0001	1-10
					160–179	1.04 (0.52–2.07)	1.99 (0.82–4.85)		
					180–199	1	1		
					200–219	0.56 (0.24–1.28)	1.09 (0.44–2.68)		
					200–239	0.49 (0.16–1.44)	0.41 (0.11–1.52)		
					> 240	-	0.80 (0.28–2.27)		
Ahn, 2009, Finland [42]	Placebo-controlled, double-blinded primary prevention trial in male smokers (ATBC)	191/29,093	18.0 years	< 203.9	< 203.9	1	-	P=0.0007	1-5, 11-17
					203.9-227.6	0.69 (0.46-1.05)	-		
					227.7-249.2	0.63 (0.41-0.97)	-		
					249.3-276.6	0.56 (0.36-0.88)	-		
					> 276.7	0.66 (0.43-1.01)	-		
Kitahara, 2011, Korea [44]	Prospective study of Korean men and women (Korean NHIC)	10,161/1,189,719	12.7 years	< 160	< 160	1	-	Men < 0.001 Women < 0.001	2-5, 13, 18
					160-179	0.69 (0.65-74)	0.63 (0.54-0.72)		
					180-199	0.62 (0.58-0.66)	0.50 (0.44-0.58)		
					200-239	0.48 (0.45-0.51)	0.37(0.32-0.42)		
					≥ 240	0.42 (0.38-0.45)	0.32 (0.27-0.39)		

JPHC Study = The Japan Public Health Center-based Prospective Study, ATBC = The Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study, Korean NHIC = The Korean National Health Insurance Corporation Medical Evaluation. *Tests for linear trend were conducted by treating the total cholesterol as a continuous variable in the multivariable models. Confounders for adjustment: 1 = age, 2 = BMI, 3 = smoking, 4 = ethanol intake, 5 = hypertension, 6 = diabetes, 7 = hyperlipidemia medication use, 8 = total vegetable intake, 9 = coffee intake, 10 = public health center, 11 = intervention, 12 = level of education, 13 = physical activity, 14 = Saturates fat intake, 15 = polyunsaturated fat intake, 16 = total calorie, 17 = serum HDL cholesterol, 18 = fasting serum glucose.

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Supplementary Table 4. Published trials of statin use as adjuvant in treatment of liver cancer

Studies	Study design	Patients population	Intervention	Control	Overall survival of intervention (months)	Overall survival of control (months)	Kaplan-Meier and log-rank test
Kawata, 2001, Japan [49]	Prospective, randomized, open label study	Patients with advanced liver cancer after TAE procedure, n = 83	Pravastatin 20-40 mg + 5-FU 200 mg QD, n = 41	5-FU 200 mg QD, n = 42	Median 18	Median 9	*P* = 0.006
Lersch, 2004, Germany [50]	Prospective study	Patients with advanced liver cancer, n = 58	Pravastatin 40-80 mg QD, n = 20	A: Octreotide, n = 30; B: Gemcitabine, n = 8	Median 7.2 (95% CIs 2.9-11.5)	A: Median 5(95% CIs 2.2-7.8);B: Median 3.5 (95% CIs 2.2-4.9)	A: *P* = 0.09; B: *P* = 0.03
Graf, 2008, Germany [51]	Prospective, non-randomized, open label study	Patients with advanced liver cancer after TACE, n = 183	Pravastatin 20-40 mg QD, n = 52	No treatment, n = 131	Median 20.9 (95% CIs 15.5-26.3)	Median 20.9 (95% CIs 15.5-26.3)	*P* = 0.003
Georgescu, 2011,Romania [52]	Prospective, randomized, open label study	Patients with advanced liver cancer, n = 72	Lovastatin 40 mg + Sorafenib 400 mg QD, n = 39	Sorafenib 400 mg QD, n = 33	Mean 12.15±0.76	Mean 10.85±0.82	Non-significant

TAE = Transcatheter Arterial Embolization; TACE = Transhepatic Arterial Chemotherapy and Embolization).

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Supplementary Table 5. Ongoing clinical trials of statin use as adjuvant in treatment of liver cancer

Studies	Year	Location	Phase	Study design	Condition	Intervention	Control	Estimated Enrollment	Resist number	Status
ESTAHEP-2010	2011	Spain	II	Multicenter, prospective, randomized, double-blind, placebo-controlled study	Advanced liver cancer	Sorafenib 400 mg BID + Pravastatin 40 mg, QD	Sorafenib 400 mg BID + placebo QD	216	NCT01418729; EUCTR2010-024421-21-ES	Recruiting
PRODIGE 21	2011	France	II	Multicenter, prospective, randomized, open label study	Liver cancer with Child-Pugh B Cirrhosis	A: Sorafenib 400 mg BID;B: Pravastatin 40 mg, QD;C: Sorafenib 400 mg BID + Pravastatin 40 mg, QD	Best supportive care	160	NCT01357486	Recruiting
JOUVE PHRCK 2009	2013	France	III	prospective, randomized, open label study	Liver cancer with Child-Pugh A Cirrhosis	Sorafenib 800 mg BID + Pravastatin 40 mg, QD	Sorafenib 800 mg BID	474	NCT01903694; NCT01075555	Recruiting

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PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	4
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	5
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	no
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	6
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	5
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	5
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	6
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	6-7
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	6-7
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	7
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	7
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	7

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PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	8
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	8
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	8
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	9 Table1,2
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	10, Suppl. Table 1
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	Figure 2
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	10
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	10
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	10-12
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	12-13
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	13-14
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	15-16
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	NA

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(6): e1000097. doi:10.1371/journal.pmed1000097

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