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[Intervention Protocol]

Tamoxifen for hepatocellular carcinoma

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ABSTRACT

Objectives

This is a protocol for a Cochrane Review (intervention). The objectives are as follows:

To assess the benefits and harms of tamoxifen in people with hepatocellular carcinoma, irrespective of sex, administered dose, type of formulation, and duration of treatment.
BACKGROUND
Description of the condition

Hepatocellular carcinoma (HCC) is the most common primary liver cancer, responsible for 70% to 85% of all people with primary liver cancer (Perz 2006; Forner 2018). The burden of liver cancer in 2017 was 20.8 million disability-adjusted life-years (DALYs) (95% uncertainty interval 19.9 million to 21.8 million) (Fitzmaurice 2019). HCC is the sixth most common type of cancer (Asrani 2019; Fitzmaurice 2019). It is the fourth leading cause of cancer-related deaths worldwide (i.e. 810,000 deaths in 2015 (Asrani 2019)), albeit with substantial variation in age, sex, and geographic distribution (Bray 2018). According to the GLOBOCAN estimate in 2018, the age-standardised incidences were highest in Eastern Asia, followed by South-Eastern Asia and Northern Africa (PETrick 2016; Bray 2018). HCC incidence is high in men aged 55 to 64 years, especially those born in the peak era of hepatitis C virus (HCV) infection (Kulik 2019). The disease burden is highest in areas with endemic hepatitis B virus (HBV) infection (where hepatitis B surface antigen (HBsAg) prevalence is ≥ 8%), such as sub-Saharan Africa and Eastern Asia, with incidence rates of over 20 per 100,000 individuals. Mediterranean countries, such as Italy, Spain, and Greece, have intermediate incidence rates of 10 to 20 per 100,000 individuals, while North and South America have a relatively low incidence (< 5 per 100,000 individuals) (Mittal 2013). Cirrhosis is an important risk factor for HCC, and among all aetiologies of cirrhosis, the risk is higher in people with chronic viral hepatitis. Overall, one-third of people with cirrhosis will develop HCC during their lifetime (EASL 2018), while around 40% of each HCC is attributed to HBV or HCV infections, 11% to alcohol consumption, and 10% to other non-specific causes (PETrick 2016; Asrani 2019). An epidemiologic study reported that the yearly cumulative incidence of HCC was 2.6% in the non-alcoholic fatty liver disease (NAFLD) group compared to 4% in the HCV group (Ascha 2010; Kawamura 2012).

Diagnosis of HCC is conventionally based on biopsy or imaging. The treatment options for people with HCC depend on tumour stages. The most widely used algorithm for patient prognosis and treatment allocation is the Barcelona Clinic Liver Cancer (BCLC) staging, which includes five stages (0, A, B, C, and D) (Forner 2012; Richani 2016). Allocation of individuals to either curative or palliative therapy is based on three main prognostic variables: tumour status (number, size, vascular invasion, extrahepatic localisation); liver function (Child-Pugh score); and performance status (defined by the Eastern Cooperative Oncology Group scale) (Richani 2016; EASL 2018). Accordingly, the curative treatment group usually includes early stage HCC (BCLC-A), and the noncurative/palliative treatment group consists of intermediate and advanced stages of HCC (BCLC-B, C), in which surgical resection and radiofrequency ablation are not considered as primary treatment modalities (Choi 2011).

Although early stage of HCC (i.e. a single lesion of less than 5 cm or up to three nodules with less than 3 cm each) is becoming more successfully managed with different treatment modalities (hepatic resection, ablative therapy, and orthotopic liver transplantation), management of advanced HCC remains challenging, especially for people with end-stage HCC, whose lesions are usually non-reatachable (Shi 2014; Ottaviano 2017). People with end-stage HCC have a very poor prognosis, with a mean survival of four to six months (Ottaviano 2017). Surgery is considered the treatment of choice for HCC, but only a small proportion of people are candidates for radical resection at the time of diagnosis. For most non-resectable individuals, the need for an effective non-surgical treatment is obvious (Simonetti 1997; Nowak 2020). There are various treatment options for unresectable HCCs, including: local ablation/radiofrequency ablation; transarterial chemoembolisation (TACE); drug-eluting bead transarterial chemoembolisation (DEB-TACE); transarterial radio embolisation (TARE); yttrium90 (Y-90); external beam radiation therapy; stereotactic body radiation therapy (SBRT); and systemic chemotherapy. Considering chemotherapeutic agents, to date sorafenib has been the only systemic chemotherapy with a proven survival benefit in HCC (Ottaviano 2017). However, there are concerns over unwanted dermatological reactions such as sorafenib-induced erythema multiforme (Namba 2012), rash/desquamation, hand-foot skin reaction, and diarrhoea (Ye 2016). Any benefit from treatment with sorafenib in HCC should, therefore, be balanced against the possible associated harms.

The remarkable sex disparity of more males than females with HCC, documented in both epidemiological studies and clinical observations, may correspond to more frequent progression of chronic liver disease to cirrhosis in males than females (Kalra 2008). Cirrhosis, presenting characteristically with hormone imbalance and a relative hyper oestrogenic state (De Maria 2002), leading to HCC development, is largely considered to affect men and postmenopausal women (Shimizu 2003; Kalra 2008). Several preclinical studies suggested androgen/androgen receptors might promote the development of aflatoxin B1-induced HCC (Pinkerton 2010). As such, it has been hypothesised that sex hormones may play a prominent role in contributing to the sex difference in HCC development (Pinkerton 2010), and hepatic regeneration in HCC (Francavilla 1989; Shi 2014). Hence, anti-oestrogen or anti-androgen agents, or both, may have potential as an adjuvant for the endocrine treatment of established HCC. In this context, tamoxifen, which is an oestrogen antagonist (Francavilla 1989; Shi 2014), as well as an anti-androgen (Vizoso 2007), is an interesting candidate for adjuvant anti-hormone therapy for HCC.

Description of the intervention

The aim of HCC treatment is to increase survival, whilst maintaining the highest quality of life. Tamoxifen is a synthetic nonsteroidal triphenylethylene and has been granted United States’ Food and Drug Administration (FDA) approval since 30 December 1977 (FDA 2002), and is listed as an essential medicine by the World Health Organization (Jordan 2011). It is one of the pioneering selective oestrogen receptor modulators that can switch target sites on tumours. Preclinical studies have shown that oestrogens support hepatocyte proliferation in vitro (Francavilla 1988). Hence, tamoxifen, which is a competitive antagonist of the oestrogen receptors (Shi 2014), can halt oestrogen and inhibit hepatocyte proliferation (FDA 2002). Breast and prostate cancers are modulated by oestrogen and androgen, respectively, while HCC may be modulated by both sex hormones during its initiation, progression, and metastasis (Shi 2014). It has been documented that both androgens and oestrogens may enhance liver carcinogenesis, while androgens may also support the growth of established liver tumours (Ma 2002).
An immunohistochemical study with resected specimens from 31 people with HCC and controls showed that around two-thirds (67.7%) were positively stained for androgen receptor (AR), 51.6% for oestrogen receptors, 83.8% for progesterone receptors, and 38.7% for apolipoprotein D (proteins that bind lipids to form lipoproteins) (Vizoso 2007).

Tamoxifen is an interesting candidate for treatment of solid tumours responding to hormonal manipulation. Following a single oral dose of 20 mg tamoxifen, an average peak plasma concentration of 40 ng/mL (range 35 ng/mL to 45 ng/mL) occurred approximately five hours after administration. A terminal elimination half-life of this drug is five to seven days (FDA 2002). Tamoxifen can be given as either monotherapy or in combination with doxorubicin, transcatheter arterial chemotherapy (TAC), TACE, luteinising hormone-releasing hormone-analogue triptorelin, and interferon (El-Serag 2011; Forner 2018).

How the intervention might work

Most HCCs are oestrogen receptor-negative, and of those with positive oestrogen receptors, many have variant oestrogen receptors (Chow 1998). For instance, a study on 111 people with HCC reported that the variant oestrogen receptors transcript was significantly overexpressed in men (P = 0.004) as well as in HBsAg-positive individuals (P = 0.0015). The significantly higher occurrence of variant oestrogen receptors in men (especially in HBsAg-positive men) at an early stage of disease suggests that the alteration of oestrogen receptors favours uncontrolled proliferation and hyperplasia by facilitating neoplastic transformation (Villa 1998; Villa 2008).

Moreover, there are various possible actions of tamoxifen, either oestrogen receptor-dependent or oestrogen receptor-independent, in inhibiting cell growth of HCCs.

Among the oestrogen receptor-dependent mechanisms, the exact pathways are not fully understood. However, based on preclinical studies, the possible mechanisms in oestrogen receptor-positive HCCs include:

- inhibition of Na+ influx, which is an early event in hepatocyte proliferation (Koch 1979; Francavilla 1989; Li 2019);
- inhibition of DNA synthesis in limited duration, not by killing hepatocytes (i.e. did not appear to be cytotoxic), but through an enhanced hepatic regenerative process, leading to full liver weight restoration at 10 days after partial hepatectomy (Francavilla 1989);
- exertion of antitumour effects by binding to oestrogen receptors (FDA 2002).

Among the oestrogen receptor-independent mechanisms, it has been suggested that the action of tamoxifen on HCC may be related to:

- protein kinase C (PKC) inhibition or calmodulin pathways that may account for tamoxifen inhibition of cell growth (Jiang 1995);
- inhibition of some intracellular biochemical reaction which might be independent of epidermal growth factor receptor interactions (Francavilla 1989).

Among androgen receptor-dependent mechanisms, it may enhance hepatic carcinogenesis by modulating cell cycle-related kinase (CCRK)-β-catenine activation signalling (Yu 2014), or promote HBV virus replication to increase viral titre, viral antigens in HBV-related HCC, leading to cellular oxidative stress (Lavarone 2003; Lipov 2009; Ivanov 2017).

A schematic presentation of how tamoxifen may inhibit cell growth in HCC is illustrated in Figure 1.

Why it is important to do this review

In a non-Cochrane systematic review, a subgroup consisting of five randomised clinical trials (RCTs), comparing tamoxifen to non-active treatment in people with HCC, showed a marginally better survival rate at one year in the tamoxifen group (odds ratio (OR) 2.0, 95% CI 1.1 to 3.6) (Simonetti 1997). There is a 1998 protocol on this topic in the Cochrane Library (Stockler 1998), and a Cochrane Review published 17 years ago (Nowak 2004). The latter included nine RCTs with a total of 1709 people with HCC, and reported a comparable effect of tamoxifen on overall survival (hazard ratio (HR) 1.05, 95% CI 0.94 to 1.16) compared with placebo or no intervention. The 2004 review has been withdrawn as it has not been updated since then (Nowak 2020). New trials have surged since the publication of those reviews, and there have been...
advances in methodology in Cochrane Reviews, such as a more robust tool for risk of bias assessment. We have not identified any systematic reviews or meta-analyses assessing the benefits and harms of tamoxifen for unresectable HCC, after the 2004 Cochrane Review (Nowak 2004).

Tamoxifen is comparatively inexpensive and may be more widely available than other interventions. Hence, if it is effective, tamoxifen may have a role as an adjunct treatment of HCC. However, pieces of evidence suggest hepatotoxicity in preclinical studies, though hormones are assumed to play a prominent role in hepatic regeneration after partial hepatectomy (Francavilla 1989; Gao 2016). Since tamoxifen can act as an agonist for uterine endometrial hyperplasia and polyp production, a possible increased risk of endometrial cancer is a concern for females (Jordan 2011). Furthermore, preclinical studies reported resistance to tamoxifen and this may be an important clinical problem, if it occurs in humans. Therefore, it is important to undertake a comprehensive assessment of all the available data on both the benefits and harms of tamoxifen in treating patients with HCC.

**OBJECTIVES**

To assess the benefits and harms of tamoxifen in people with hepatocellular carcinoma, irrespective of sex, administered dose, type of formulation, and duration of treatment.

**METHODS**

**Criteria for considering studies for this review**

**Types of studies**

We will include randomised clinical trials (RCTs) with a parallel-group design. We will include the first phase of cross-over trials, but we will exclude the second phase because of the potential ‘residual effect’ regarding the metabolite of tamoxifen.

We will not meta-analyse data from studies labelled as ‘quasi-randomised’ (i.e. pseudo-randomised). However, if during the search and selection of trials, we identify observational studies (e.g. cohort studies or case reports) that have reported adverse events associated with tamoxifen, we will include these studies for a review of the reported adverse events only. We will present these data separately. We will not specifically search for observational studies for inclusion in this review, which is a limitation. We are conscious that by not looking for all observational studies on adverse events, we allow the risks of putting more weight on potential benefits than on potential harms, and of overlooking uncommon and late adverse events (Storebø 2018).

**Types of participants**

We will include adults (people over 18 years of age) of any sex, diagnosed with HCC of any stage. If we find trials evaluating people with both HCC and non-HCC disease, then we will include only those participants with HCC disease from which data could be extracted separately.

**Types of interventions**

**Experimental intervention**

- Tamoxifen
- Tamoxifen administered with any other anticancer drug

**Control intervention**

- Placebo or no intervention
- Standard care (i.e. systemic chemotherapy, surgical resection, liver transplantation, transarterial chemoembolisation)
- Alternative therapy (e.g. genistein administered together with other anticancer drugs)

Co-interventions will be allowed if used equally in the experimental and control groups of a trial.

We will include all types of tamoxifen, regardless of dosage, schedule, formulation (e.g. pills or liquid) or the source of manufacturers.

**Types of outcome measures**

Our primary analysis, on which we will base our main conclusion, will include data at the longest follow-up because it is clinically most relevant. We also aim to assess all outcomes at 12 months, 24 months, 36 months, and longer than 36 months.

**Primary outcomes**

- Proportion of people with all-cause mortality
- Proportion of people with one or more serious adverse events if the event(s):
  
  * fulfills the definition of serious adverse events of the International Conference on Harmonisation (ICH) Guidelines (ICH-GCP 1997). Namely, any untoward medical occurrence that: leads to death; is life-threatening; requires in-patient hospitalisation or prolongation of existing hospitalisation; results in persistent or significant disability; is a congenital anomaly or birth defect; and any important medical event that may have jeopardised the individual or required intervention to prevent it (ICH-GCP 1997); or
  
  * is defined as a serious adverse event by the trial investigators.

  We will consider all other adverse events as non-serious.

- Health-related quality of life (measured with validated questionnaires (e.g. the World Health Organization’s (WHO) quality of life questionnaire (WHOQOL); the European quality of life measure (EuroQol); the 36-item Short Form Health Survey (SF-36)).

**Secondary outcomes**

- Proportion of people with disease (HCC) progression (as defined in the trial)
- Proportion of people with one or more adverse events considered non-serious, or not included in the definition for serious adverse events
- Proportion of people without improvement in liver function tests (e.g. unchanged or increased activity of alanine amino transferase (ALT) or aspartate amino transferase (AST)).

**Search methods for identification of studies**

**Electronic searches**

We will search the Cochrane Hepato-Biliary Group (CHBG) Controlled Trials Register (maintained and searched internally by the CHBG Information Specialist via the Cochrane Register of Studies Web; date of search will be given at review stage); the Cochrane Central Register of Controlled Trials (CENTRAL;
We will use a data collection form for study characteristics and outcome data, which has been piloted on at least one trial in the review. One review author (CN) will extract the following study characteristics and outcome data from included trials.

- **Methods:** study design, study period, study centres and location, study setting, withdrawals/dropouts, and date of study.
- **Participants:** mean age, age range, sex, diagnostic methods, severity of condition (e.g. Cancer of Liver Italian Program (CLIP) score, Barcelona Clinic Liver Cancer (BCLC) staging), inclusion criteria and exclusion criteria.
- **Interventions:** intervention, comparison, concomitant medications, and excluded medications.
- **Outcomes:** planned outcomes in the trial protocol, if available, for later comparison during risk of bias assessment.
- **Time points of the outcome data.**
- **Notes:** funding for studies and notable conflicts of interest of trial authors.

We will not include outcomes using time-to-event data.

For dichotomous outcomes (e.g. serious adverse events), we will extract the number of participants in each arm who experienced the outcome of interest, and the number of participants assessed at a certain time point in order to estimate a risk ratio (RR).

For continuous outcomes (e.g. quality of life measures), we will extract the mean difference (MD) and standard deviation (SD) between the value of the outcome measure in each trial arm at the end of follow-up. If the SDs are not available, we will use change scores if their SDs are available. If no SDs are available, we will omit these trials from the analyses and include them only in a narrative analysis with a table.

Two review authors (HN and CN) will independently extract outcome data from the included studies. We will note in the ‘Characteristics of included studies’ tables if outcome data were not reported in a usable way. We will resolve disagreements by consensus involving all authors.

One review author (CN) will do the data entry into ‘Characteristics of included studies’ tables in Review Manager Web (RevMan Web 2020). Another review author (HN) will check trial characteristics for accuracy against the trial report.

### Assessment of risk of bias in included studies

Two review authors (CN and HN) will independently assess the risk of bias in the included studies. We will resolve any disagreement by consensus, or if required, by consulting a third review author (HHA).

We will assess risk of bias (RoB) using the RoB 2 tool (Sterne 2019; Higgins 2021a). We will assess the effect of assignment to the intervention (Higgins 2021a). To be able to do this, we will use the intention-to-treat (ITT) principle. ITT includes all randomised participants, regardless of the interventions they actually received.

We will use these five domains to assess risk of bias in the individually randomised trials (Higgins 2021a; Higgins 2021b):

- bias arising from the randomisation process;
- bias due to deviations from intended interventions;
- bias due to missing outcome data;
- bias in measurement of an outcome; and
- bias in selection of the reported result.

We provide the signalling questions for these domains in Appendix 2. The response options for the signalling questions are ‘Yes’, ‘Probably yes’, ‘No’, ‘Probably no’, and ‘No information’. Elaborations to these signalling questions can be found in Higgins 2021a. An algorithm, developed in Microsoft Excel, will map our responses to the signalling questions for each outcome and will propose a risk of bias judgement for each of the domains.
Algorithms for the judgement of bias arising from each domain is provided in Higgins 2019.

If we identify trials with a cross-over design, then there is an additional consideration of the possibility that first period data are selected. We will include this signalling question for the process of randomisation: ‘Did baseline differences between intervention groups at the start of the first period suggest a problem with the randomisation process?’ (Higgins 2020; Higgins 2021b). An algorithm for reaching risk of bias judgements in a cross-over trial is provided in Figure 2.

**Figure 2. Algorithm for risk of bias judgements for bias arising from the randomisation process in a cross-over trial**

We will assign one of the three levels of judgement to each domain as indicated below:

- **low risk of bias:** the trial is judged to be at low risk of bias for all domains for this result;
- **some concerns:** the trial is judged to raise some concerns in at least one domain for this result, but is not at high risk of bias for any of the remaining domains;
- **high risk of bias:** the trial is judged to be at high risk of bias in at least one domain for this result or the study is judged to have some concerns for multiple domains in a way that substantially lowers confidence in the result.

The overall risk of bias judgement can be the same as for the individual domains; namely, low risk of bias, some concerns, or high risk of bias. Judging a result to be at a particular level of risk of bias for an individual domain implies that the result has an overall risk of bias at least this severe. **Figure 3** illustrates how we will reach an overall judgment of risk of bias.
Figure 3. Domains of bias assessment
• Domain 1: bias arising from the randomisation process
• Domain 2: bias due to deviations from intended interventions
• Domain 3: bias due to missing outcome data
• Domain 4: bias in measurement of the outcome
• Domain 5: bias in selection of the reported result

We will use the RoB 2 Microsoft Excel tool to store the data (which can be received by request) until we find a place to make them publicly available.

The RoB 2 assessments will feed into one domain of the GRADE approach for assessing the certainty of a body of evidence (Schünemann 2021a).

We will focus on outcome results of the trials that contribute information which we anticipate that users of the review will find most useful. Therefore, in summary of findings (SoF) tables, we will present the outcome results of risk of bias for i) all-cause mortality at maximum follow-up, ii) proportion of people with one or more serious adverse events at maximum follow-up, iii) health-related quality of life at maximum follow-up, iv) proportion of people with disease progression at maximum follow-up, and v) proportion of people with one or more adverse events considered non-serious, or not included in the definition for serious adverse events at maximum follow-up.

Measures of treatment effect
We will report dichotomous outcomes using the risk ratio (RR) and its 95% CI to report outcomes when different scales are used to measure the same outcome. We will interpret SMD as follows: SMD less than 0.40 for small intervention effects; SMD between 0.40 and 0.70 for moderate intervention effects; and SMD greater than 0.70 for large intervention effects, as described in Chapter 15.5.3.1 of the Cochrane Handbook for Systematic Reviews of Interventions (Schünemann 2021b, hereafter referred to as the Cochrane Handbook). We will describe in a narrative form skewed data reported as medians and interquartile ranges as described in Chapter 10.5.3 of the Cochrane Handbook (Deeks 2021).

We will not report the hazard ratio (HR) and its 95% CI as we will not include outcomes using time-to-event data. If time-to-event outcome data are provided as dichotomous data at a fixed time point (e.g. at least 12 months), we will also construct a 2 × 2 table and we will express intervention effects as RR (Higgins 2021c). We will estimate the ‘overall effect’ across all outcomes.

Unit of analysis issues
In trials with individually randomised, parallel group design, the unit of analysis will be the trial participant as randomised within the trial. We do not expect to find cluster-randomised trials, but if we do find such trials, the unit of analysis will be the groups of participants (e.g. schools, villages, medical practices, patients.
of a single doctor, or families) as randomised (Higgins 2021c). We will not combine the data from cluster-randomised trials with individually randomised trials in the same meta-analysis (i.e. they will be analysed separately).

In trials with a cross-over design, we will only include data from the first trial period because the half-life of tamoxifen is 5 days to 7 days (FDA 2002), and it is likely to accumulate following multiple subcutaneous doses.

For dichotomous outcomes (e.g. presence/absence of a serious adverse event), we will use participants as unit of analysis, rather than events (i.e. the number of participants with a hospital admission rather than the number of admissions per participant). However, if a trial reports rate ratios, we will analyse them on the basis of events rather than participants. Where a single trial reports multiple trial intervention groups, we will include only the relevant groups for our comparison. If we combine two comparison groups in the same meta-analysis (e.g. AA versus BB, and CC versus BB), we will halve the control group (i.e. BB) to avoid double-counting. We will record whether the trial measures outcomes (e.g. adverse events) in relation to frequency of participants with an adverse event (e.g. three participants reported vomiting), or to multiple adverse events in the same participant (e.g. one participant had three episodes of vomiting). We will also record occasions where multiple events in a participant have been incorrectly treated as independent without taking into account the interdependence of the events. Where the number of events appear to be equal to the number of participants, we will treat the events as the unit of analysis as described in Chapter 6 of the Cochrane Handbook (Higgins 2021c).

Dealing with missing data
We will contact investigators or study sponsors in order to verify key study characteristics and obtain missing numerical outcome data where possible (e.g. when we identify a study published only as an abstract). Where this is not possible, and we think the missing data could introduce serious bias, we will take this into consideration in the GRADE rating for affected outcomes.

We will perform an intention-to-treat analysis whenever possible (Newell 1992). If there are missing standard deviations (SDs) for continuous outcomes, we will contact the corresponding trial authors to see if data are available. If not available, we plan to calculate these using case-analysis such as imputing SDs from standard errors (SEs), CIs, t-values, or P values (as appropriate) that relate to the differences between means in two groups, following the guidance described in Chapter 6.5.2 of the Cochrane Handbook (Higgins 2021c). When there is insufficient information to calculate the SDs, we plan to impute them. We plan to replace missing SDs for ‘change from baseline’ with those provided in other trials for the same outcome. If this approach is not applicable, assuming that correlation coefficients from the two intervention groups are similar, we may impute a SD of the change from baseline for the experimental intervention, following a formula as described in the Cochrane Handbook (Deeks 2021).

Assessment of heterogeneity

Based on the study characteristics, including study design, population, and details on the interventions, we will describe the clinical diversity and methodological variability of the evidence in our review. We will interpret statistical heterogeneity with the Chi² test, included in the forest plots. We will use a P value of less than 0.10 to indicate statistical heterogeneity, as described in Chapter 10.10.2 of the Cochrane Handbook (Deeks 2021), and we will quantify heterogeneity using the I² statistic if the P value is less than 0.10.

Using the I² statistic, we will measure the heterogeneity among the trials in each analysis, and we will interpret it as in Deeks 2021:

- 0% to 40%: might not be important;
- 30% to 60%: may represent moderate heterogeneity;
- 50% to 90%: may represent substantial heterogeneity;
- 75% to 100%: considerable heterogeneity.

If we identify substantial heterogeneity (I² > 50%), we will report it and explore the possible causes by prespecified subgroup analyses. We will use the random-effects model meta-analysis to account for the presence of between-study heterogeneity.

Assessment of reporting biases
If we are able to analyse at least 10 trials in one meta-analysis, we will create and examine a funnel plot to explore possible small study and publication biases (Deeks 2021). We will stratify the funnel plots by risk of bias if at least ten trials are found for each level of bias (Sterne 2019).

Data synthesis
If there are at least two clinically similar trials, we will meta-analyse their results. We will use a random-effects model (DerSimonian 1986). As we expect to gather data from a series of trials performed by different researchers operating independently, it is unlikely that all the trials will be functionally equivalent with a common effect estimate. Therefore, the random-effects model is more appropriate than the fixed-effect model. We will use the fixed-effect model as a sensitivity analysis (DeMets 1987). We will present all results with 95% CIs. We will conduct all analyses according to the guidance provided in the Cochrane Handbook (Deeks 2021), and implement it in Review Manager Web (RevMan Web 2020).

We will include trials at any risk of bias in our primary analysis. However, depending on the number of the included trials and the number of trials at low risk of bias, the number of trials at low risk of bias and at some concern for risk of bias, we may also do sensitivity analysis (Boutron 2021).

We may not do meta-analysis if there is considerable heterogeneity that we cannot explain, or trials report outcomes differently (e.g. impossible to calculate the same effect measure from the available statistics) as described in Chapter 12 (Table 12.1.a) in the Cochrane Handbook (McKenzie 2021). In this scenario, we will summarise the main findings and results of the included trials in a narrative format.

Subgroup analysis and investigation of heterogeneity

In the event of substantial clinical, methodological (trials at ‘high’ risk of bias compared to those at ‘low’ risk of bias or ‘some concerns’), or statistical heterogeneity, we will attempt to find the possible reasons for heterogeneity by evaluating the individual trials and their subgroup characteristics. We also plan to carry out the following subgroup analyses, regardless of the presence of heterogeneity, if we have enough trial data.
• Trials at 'low' risk of bias compared to trials at 'some concern' or at 'high' risk of bias (because the latter trials may overestimate beneficial intervention effects or underestimate harmful intervention effects) (Schulz 1995; Moher 1998; Hjärgard 2001; Wood 2008; Savović 2012a; Savović 2012b; Savović 2018; Higgins 2021a).

• Trials at risk of for-profit support compared to trials without for-profit support (because trials with for-profit support may overestimate beneficial intervention effects or underestimate harmful intervention effects) (Lundh 2017).

• Sex (males compared to females) because sex-specific hormones (testosterone in males and oestrogen in women) may influence the treatment outcomes. This natural discrepancy is likely to obtain with tamoxifen, which acts on androgen/ oestrogen receptors.

• Stages of HCC (e.g. early stage compared to advanced stage (because advanced stage HCC has more tumour burden (larger tumour) than earlier HCC stages). Tamoxifen may be less effective in expected shrinkage of a large tumour compared to a small tumour (i.e. early stage). This is because the risk of vascular invasion and dissemination increases with the diameter of a tumour (Fuke 2012).

• Pre-existing cirrhosis compared to non-cirrhotic liver (because most HCC is progressed from pre-existing cirrhosis while some HCCs are not. This difference can affect the outcomes of tamoxifen).

• Pre-treatment status (e.g. treatment-naïve participants or pre-treated participants with HCC; alternative prior treatment) may influence the treatment outcomes. Some HCCs progress because of chronic hepatitis infections. Therefore, a successful treatment of viral hepatitis will facilitate subsequent tamoxifen therapy in HCC. A large proportion of people with HCC die from complications of liver cirrhosis and portal hypertension (i.e. gastrointestinal bleeding, infections, renal failure) rather than from clearly tumour-related causes (Pinter 2016). Hence, adequate evaluation and treatment of portal hypertension can reduce HCC-related mortality. Also, prior treatment with a potent nucleoside/nucleotide analogue (NAs) could produce suppressive effects on HCC recurrence (Chuma 2009). Hence, the difference in status of prior treatment can affect the outcomes of tamoxifen.

If there are sufficient studies to make subgroup comparisons meaningful, we will use a formal statistical approach to examine differences among subgroups, as described in Chapter 10.11.3.1 of the Cochrane Handbook (Deeks 2021).

**Sensitivity analysis**

We plan to carry out these sensitivity analyses for the primary outcomes:

- excluding trials at some concern and at high risk of bias;
- performing analysis with a fixed-effect model; and
- assessment of imprecision with Trial Sequential Analysis (see below).

**Trial Sequential Analysis**

We will use Trial Sequential Analysis (Jakobsen 2014; Castellini 2018; Gartlehner 2019) as sensitivity analysis of the evaluation of imprecision with GRADE. We will perform Trial Sequential Analysis using the following outcomes at maximum follow-up: all-cause mortality, proportion of people with one or more serious adverse events, health-related quality of life, proportion of people with disease progression, and proportion of people with one or more adverse events considered non-serious, or not included in the definition for serious adverse events at maximum follow-up. In Trial Sequential Analysis, we will downgrade our assessment of imprecision by two levels if the accrued number of participants is below 50% of the diversity-adjusted required information size (DARIS), and one level if it is between 50% and 100% of DARIS. We will not downgrade if trial monitoring boundaries for benefit, harm, futility, or DARIS are reached.

We plan to perform Trial Sequential Analysis in order to calculate the cumulative sample size of the meta-analysis (information size) and to reduce the risk of random errors due to sparse data and repetitive testing of accumulating data (Wetterslev 2008; Thorlund 2011; Thorlund 2017; TSA 2017). We will calculate the information size adjusted for heterogeneity (diversity) (DARIS) between trials using the following parameters (Wetterslev 2009): proportion of events in the control group estimated from the included trials (overall mean value); anticipated intervention effect (relative risk reduction, RRR) of 15%; alpha of 1.60%, as we use five outcomes; and beta of 10% (Jakobsen 2014; Wetterslev 2017). For the continuous outcome, health-related quality of life, we will use a minimal relevant difference equal to standard deviation (SD)/2; SD of the control group; alpha of 1.60%; beta of 10%; and diversity of the meta-analysis. We will add trials to the analysis according to the year of publication. If more than one trial is published in a year, we will add the trials in alphabetical order, according to the year of the first author. On the basis of the required information size, we will construct the trial sequential monitoring boundaries for benefits, harms, and futility using the Lan-DeMets O'Brien-Fleming alpha-spending and beta-spending functions. The boundaries for benefit and harms are used for meta-analyses that have not reached the required information size to conclude when statistical significance is reached. If the trial sequential monitoring boundary is crossed before the required information size is reached, a sufficient level of evidence is reached, results of the meta-analysis can be considered conclusive if bias can be excluded, and no additional trials may be needed. Conversely, if the boundary is not crossed, the meta-analysis is inconclusive, and more trials may be needed to detect or reject a certain intervention effect. When the cumulative Z-curve crosses the futility boundaries, a sufficient level of evidence is reached that the two treatments do not differ by more than 15% (anticipated intervention effect used in information size estimation), and no additional trials may be needed. In all situations where no trial sequential monitoring boundaries are reached, further trials may be needed until the information size is reached, or until monitoring boundaries are crossed.

**Summary of findings and assessment of the certainty of the evidence**

We will use summary of findings tables to present results of the review comparisons. Two review authors (HN, CN) will apply GRADE criteria and will resolve any disagreements by discussion. If disagreements are not resolved, then the senior author (JWM) will arbitrate. We will use the five GRADE domains - i.e. risk of bias (we will use the overall RoB 2 judgement), consistency of effect, imprecision (calculating also the optimal information size), indirectness, and publication bias - to assess the certainty.
of evidence in the trials' outcome data results. We will use the methods and recommendations described in Chapter 14 of the Cochrane Handbook (Schünemann 2021a), using GRADEpro GDT software (GRADEpro GDT). We will justify all decisions to downgrade the quality of the trials using footnotes, and we will make comments to aid the reader's understanding of the review where necessary.

The levels of evidence are defined as 'high', 'moderate', 'low', or 'very low'. These grades are defined as follows.

- **High certainty**: we are very confident that the true effect lies close to that of the estimate of the effect.
- **Moderate certainty**: we are moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.
- **Low certainty**: our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect.
- **Very low certainty**: we have very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of the effect.

The summary of findings tables will include the previously mentioned outcomes at maximum follow-up: i) all-cause mortality, ii) proportion of people with one or more serious adverse events, iii) health-related quality of life, iv) proportion of people with disease progression, and v) proportion of people with one or more adverse events considered non-serious, or not included in the definition for serious adverse events. We will provide the maximum time period of follow-up (mean/median and range).

**ACKNOWLEDGEMENTS**

The authors thank Dimitrinka Nikolova, Sarah Louise Klingenberg, and Christian Gluud from the Cochrane Hepato-Biliary Group Editorial Team Office for assistance in updating the initial protocol and developing new trial search strategies. We thank Norah Htet Htet for assistance in figure illustrations.

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Peer Reviewers: Laura Dawson, Canada; Kerry Dwan, UK
Contact Editors: Rosa Simonetti, Denmark; Christian Gluud, Denmark
Sign-off Editor: Lise Lotte Gluud, Denmark
Network Editor: Rachel Richardson, UK

Deeks 2021

De Maria 2002

DeMets 1987

DerSimonian 1986

EASL 2018

El-Serag 2011

FDA 2002

Fitzmaurice 2019

Forner 2012

Forner 2018
Francavilla 1989

Fuke 2012

Gao 2016

Gartlehner 2019

GRADEpro GDT [Computer program]

Higgins 2019

Higgins 2020

Higgins 2021a

Higgins 2021b

Higgins 2021c

ICH-GCP 1997

Ivanov 2017

Jakobsen 2014

Jiang 1995

Jordan 2007

Jordan 2011

Kalra 2008

Kawamura 2012

Kjaergard 2001
Kjaergard LL, Villumsen J, Gluud C. Reported methodologic quality and discrepancies between large and small...

Koch 1979

Kulik 2019

Mittal 2013

Moher 1998

Moher 2009

Mittal 2013

Moher 1999

Mittal 2013

Moher 1998

Mittal 2013

Moher 1999

Mittal 2013

Moher 1999

Mittal 2013

Wetterslev 2009

Wetterslev 2017

Wood 2008

Ye 2016

References to other published versions of this review

Nowak 2004

Nowak 2020

Stockler 1998

APPENDICES

Appendix 1. Search strategies

<table>
<thead>
<tr>
<th>Database</th>
<th>Time span</th>
<th>Search strategy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cochrane Hepato Biliary Controlled Trials Register</td>
<td>Date of search will be given at review stage.</td>
<td>(tamoxifen or nolvadex or soltamox or tamosin or tamodex or oncotam or oncomox or mamofen or caditam) and (((liver or hepato*) and (carcinom* or cancer* or neoplasm* or malign* or tumo*)) or HCC)</td>
</tr>
<tr>
<td>Cochrane Central Register of Controlled Trials (CENTRAL) in the Cochrane Library</td>
<td>Latest issue</td>
<td>#1 MeSH descriptor: [Tamoxifen] explode all trees</td>
</tr>
<tr>
<td></td>
<td></td>
<td>#2 (tamoxifen or nolvadex or soltamox or tamosinor tamodex or oncotam or oncomox or mamofen or caditam)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>#3 #1 or #2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>#4 MeSH descriptor: [Carcinoma, Hepatocellular] explode all trees</td>
</tr>
<tr>
<td></td>
<td></td>
<td>#5 MeSH descriptor: [Liver Neoplasms] explode all trees</td>
</tr>
<tr>
<td></td>
<td></td>
<td>#6 (((liver or hepato*) and (carcinom* or cancer* or neoplasm* or malign* or tumo*)) or HCC)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>#7 #4 or #5 or #6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>#8 #3 and #7</td>
</tr>
<tr>
<td>MEDLINE Ovid</td>
<td>1946 to the date of the search</td>
<td>1. exp Tamoxifen/</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2. (tamoxifen or nolvadex or soltamox or tamosin or tamodex or oncotam or oncomox or mamofen or caditam).mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword]</td>
</tr>
</tbody>
</table>
heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]

3. 1 or 2
4. exp Carcinoma, Hepatocellular/
5. exp Liver Neoplasms/

6. (((liver or hepato*) and (carcinom* or cancer* or neoplasm* or malign* or tumor*)) or HCC).mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]

7. 4 or 5 or 6
8. 3 and 7

9. (randomized controlled trial or controlled clinical trial).pt. or clinical trials as topic.sh. or trial.ti.
10. (random* or blind* or placebo* or meta-analys*).mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]

11. 8 and (9 or 10)

EMBASE Ovid 1974 to the date of the search

1. exp tamoxifen/
2. (tamoxifen or nolvadex or soltamox or tamosin or tamodex or oncotam or oncomox or mamofen or caditam).mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word]

3. 1 or 2
4. exp liver cell carcinoma/
5. exp liver tumor/

6. (((liver or hepato*) and (carcinom* or cancer* or neoplasm* or malign* or tumor*)) or HCC).mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word]

7. 4 or 5 or 6
8. 3 and 7

9. Randomized controlled trial/ or Controlled clinical study/ or trial.ti.
10. (random* or blind* or placebo* or meta-analys*).mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word]

11. 8 and (9 or 10)
(Continued)

LILACS (Bireme) 1982 to the date of the search (tamoxifen or nolvadex or soltamox or tamosin or tamodex or oncotam or oncomox or mamofen or caditam) [Words] and (((liver or hepato$) and (carcinom$ or cancer$ or neoplasm$ or malign$ or tumo$)) or HCC) [Words]

Science Citation Index Expanded (Web of Science) 1900 to the date of the search #5 #4 AND #3
#4 TI=(random* or blind* or placebo* or meta-analys* or trial*) OR TS=(random* or blind* or placebo* or meta-analys*)
#3 #2 AND #1
#2 TS=(((liver or hepato*) and (carcinom* or cancer* or neoplasm* or malign* or tumo*)) or HCC)
#1 TS=(tamoxifen or nolvadex or soltamox or tamosin or tamodex or oncotam or oncomox or mamofen or caditam)

Conference Proceedings Citation Index - Science (Web of Science) 1990 to the date of the search #5 #4 AND #3
#4 TI=(random* or blind* or placebo* or meta-analys* or trial*) OR TS=(random* or blind* or placebo* or meta-analys*)
#3 #2 AND #1
#2 TS=(((liver or hepato*) and (carcinom* or cancer* or neoplasm* or malign* or tumo*)) or HCC)
#1 TS=(tamoxifen or nolvadex or soltamox or tamosin or tamodex or oncotam or oncomox or mamofen or caditam)

Appendix 2. Descriptions of the bias domains in RoB 2 tool for randomised trials with a summary of the issues addressed

<table>
<thead>
<tr>
<th>Bias domain</th>
<th>Issues addressed*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bias arising from the randomisation process</td>
<td>Whether:</td>
</tr>
<tr>
<td></td>
<td>• the allocation sequence was random;</td>
</tr>
<tr>
<td></td>
<td>• the allocation sequence was adequately concealed;</td>
</tr>
<tr>
<td></td>
<td>• baseline differences between intervention groups suggest a problem with the randomisation process.</td>
</tr>
<tr>
<td>Bias due to deviations from intended interventions</td>
<td>Whether:</td>
</tr>
<tr>
<td></td>
<td>• participants were aware of their assigned intervention during the trial;</td>
</tr>
<tr>
<td></td>
<td>• carers and people delivering the interventions were aware of participants’ assigned intervention during the trial.</td>
</tr>
<tr>
<td></td>
<td><em>When the review authors’ interest is in the effect of assignment to intervention (see Section 8.2.2):</em></td>
</tr>
<tr>
<td></td>
<td>• (if applicable) deviations from the intended intervention arose because of the experimental context (i.e. do not reflect usual practice); and, if so, whether they were unbalanced between groups and likely to have affected the outcome;</td>
</tr>
<tr>
<td></td>
<td>• an appropriate analysis was used to estimate the effect of assignment to intervention; and, if not, whether there was potential for a substantial impact on the result.</td>
</tr>
<tr>
<td></td>
<td><em>When the review authors’ interest is in the effect of adhering to intervention (see Section 8.2.2):</em></td>
</tr>
</tbody>
</table>
Bias due to missing outcome data

Whether:
- data for this outcome were available for all, or nearly all, participants randomized;
- (if applicable) there was evidence that the result was not biased by missing outcome data;
- (if applicable) missingness in the outcome was likely to depend on its true value (e.g. the proportions of missing outcome data, or reasons for missing outcome data, differ between intervention groups).

Bias in measurement of the outcome

Whether:
- the method of measuring the outcome was inappropriate;
- measurement or ascertainment of the outcome could have differed between intervention groups;
- outcome assessors were aware of the intervention received by study participants;
- (if applicable) assessment of the outcome was likely to have been influenced by knowledge of intervention received.

Bias in selection of the reported result

Whether:
- the trial was analysed in accordance with a pre-specified plan that was finalised before unblinded outcome data were available for analysis;
- the numerical result being assessed is likely to have been selected, on the basis of the results, from multiple outcome measurements within the outcome domain;
- the numerical result being assessed is likely to have been selected, on the basis of the results, from multiple analyses of the data.

* For the precise wording of signalling questions and guidance for answering each one, see the full risk of bias tool at www.riskofbias.info.

CONTRIBUTIONS OF AUTHORS

CN: developed the protocol with suggestions from team members.
HN: commented on the protocol. HN will provide methodological oversight to standardise the review and the contents of the review.
HHA: commented on the protocol. HHA will provide methodological oversight to standardise the review.
JWM: conceived the idea for the review and commented on the protocol. JWM will provide the contents of the review.

Text in this protocol may overlap with other protocols for Cochrane Reviews and Cochrane Reviews. This is because all protocols and reviews follow Cochrane methodology. Text in “Gene therapy for people with hepatocellular carcinoma” (CN, HHA) and “Thymosin-α1 for people with chronic hepatitis B” (CN) may overlap as the reviews have common authors.

DECLARATIONS OF INTEREST

Cho Naing: none known
Han Ni: none known
Htar Htar Aung: none known
Joon Wah Mak: none known

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  Support to CN

**External sources**

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