Cochrane Library

Cochrane Database of Systematic Reviews

Gene therapy for people with hepatocellular carcinoma (Review)

Naing C, Ni H, Aung HH, Htet NH, Nikolova D

Naing C, Ni H, Aung HH, Htet NH, Nikolova D.
Gene therapy for people with hepatocellular carcinoma.
Cochrane Database of Systematic Reviews 2024, Issue 6. Art. No.: CD013731.
DOI: 10.1002/14651858.CD013731.pub2.

www.cochranelibrary.com

WILEY

TABLE OF CONTENTS

[Intervention Review]

Gene therapy for people with hepatocellular carcinoma

Cho Naing[1], Han Ni[2], Htar Htar Aung[3], Norah Htet Htet[3], Dimitrinka Nikolova[4]

[1]Division of Tropical Health and Medicine, James Cook University, Queensland, Australia. [2]Department of Medicine, Newcastle University Medicine Malaysia, Johor, Malaysia. [3]School of Medicine, IMU University, Kuala Lumpur, Malaysia. [4]Cochrane Hepato-Biliary Group, Copenhagen Trial Unit, Centre for Clinical Intervention Research, The Capital Region, Copenhagen University Hospital — Rigshospitalet, Copenhagen, Denmark

Contact: Cho Naing, cho3699@gmail.com.

Editorial group: Cochrane Hepato-Biliary Group.
Publication status and date: Edited (no change to conclusions), published in Issue 7, 2024.

Citation: Naing C, Ni H, Aung HH, Htet NH, Nikolova D. Gene therapy for people with hepatocellular carcinoma. *Cochrane Database of Systematic Reviews* 2024, Issue 6. Art. No.: CD013731. DOI: 10.1002/14651858.CD013731.pub2.

ABSTRACT

Background

Hepatocellular carcinoma is the most common type of liver cancer, accounting for 70% to 85% of individuals with primary liver cancer. Gene therapy, which uses genes to treat or prevent diseases, holds potential for treatment, especially for tumours. Trials on the effects of gene therapy in people with hepatocellular carcinoma have been published or are ongoing.

Objectives

To evaluate the benefits and harms of gene therapy in people with hepatocellular carcinoma, irrespective of sex, administered dose, and type of formulation.

Search methods

We identified randomised clinical trials through electronic searches in The Cochrane Hepato-Biliary Group Controlled Trials Register, CENTRAL, MEDLINE, Embase, LILACS, Science Citation Index Expanded, and Conference Proceedings Citation Index–Science. We searched five online clinical trial registries to identify unpublished or ongoing trials. We checked reference lists of the retrieved studies for further trials. The date of last search was 20 January 2023.

Selection criteria

We aimed to include randomised clinical trials assessing any type of gene therapy in people diagnosed with hepatocellular carcinoma, irrespective of year, language of publication, format, or outcomes reported.

Data collection and analysis

We followed Cochrane methodology and used Review Manager to prepare the review. The primary outcomes were all-cause mortality/overall survival (whatever data were provided), serious adverse events during treatment, and health-related quality of life. The secondary outcomes were proportion of people with disease progression, adverse events considered non-serious, and proportion of people without improvement in liver function tests. We assessed risk of bias of the included trials using RoB 2 and the certainty of evidence using GRADE. We presented the results of time-to-event outcomes as hazard ratios (HR), dichotomous outcomes as risk ratios (RR), and continuous outcomes as mean difference (MD) with their 95% confidence intervals (CI). Our primary analyses were based on intention-to-treat and outcome data at the longest follow-up.

Main results

We included six randomised clinical trials with 364 participants. The participants had unresectable (i.e. advanced inoperable) hepatocellular carcinoma. We found no trials assessing the effects of gene therapy in people with operable hepatocellular carcinoma.

Four trials were conducted in China, one in several countries (from North America, Asia, and Europe), and one in Egypt. The number of participants in the six trials ranged from 10 to 129 (median 47), median age was 55.2 years, and the mean proportion of males was 72.7%. The follow-up duration ranged from six months to five years. As the trials compared different types of gene therapy and had different controls, we could not perform meta-analyses. Five of the six trials administered co-interventions equally to the experimental and control groups. All trials assessed one or more outcomes of interest in this review. The certainty of evidence was very low in five of the six comparisons and low in the double-dose gene therapy comparison. Below, we reported the results of the primary outcomes only.

Pexastimogene devacirepvec (Pexa-Vec) plus best supportive care versus best supportive care alone

There is uncertainty about whether there may be little to no difference between the effect of Pexa-Vec plus best supportive care compared with best supportive care alone on overall survival (HR 1.19, 95% CI 0.78 to 1.82; 1 trial (censored observation at 20-month follow-up), 129 participants; very low-certainty evidence) and on serious adverse events (RR 1.42, 95% CI 0.60 to 3.33; 1 trial at 20 months after treatment, 129 participants; very low-certainty evidence). The trial reported quality of life narratively as "assessment of quality of life and time to symptomatic progression was confounded by the high patient dropout rate."

Adenovirus-thymidine kinase with ganciclovir (ADV-TK/GCV) plus liver transplantation versus liver transplantation alone

There is uncertainty about whether ADV-TK/GCV plus liver transplantation may benefit all-cause mortality at the two-year follow-up (RR 0.39, 95% CI 0.20 to 0.76; 1 trial, 45 participants; very low-certainty evidence). The trial did not report serious adverse events other than mortality or quality of life.

Double-dose ADV-TK/GCV plus liver transplantation versus liver transplantation alone

There is uncertainty about whether double-dose ADV-TK/GCV plus liver transplantation versus liver transplantation may benefit all-cause mortality at five-year follow-up (RR 0.40, 95% CI 0.22 to 0.73; 1 trial, 86 participants; low-certainty evidence). The trial did not report serious adverse events other than mortality or quality of life.

Recombinant human adenovirus-p53 with hydroxycamptothecin (rAd-p53/HCT) versus hydroxycamptothecin alone

There is uncertainty about whether there may be little to no difference between the effect of rAd-p53/HCT versus hydroxycamptothecin alone on the overall survival at 12-month follow-up (RR 3.06, 95% CI 0.16 to 60.47; 1 trial, 48 participants; very low-certainty evidence). The trial did not report serious adverse events or quality of life.

rAd-p53/5-Fu (5-fluorouracil) plus transarterial chemoembolisation versus transarterial chemoembolisation alone

The trial included 46 participants. We had insufficient data to assess overall survival. The trial did not report serious adverse events or quality of life.

E1B-deleted (dl1520) adenovirus versus percutaneous ethanol injection

The trial included 10 participants. It did not report data on overall survival, serious adverse events, or health-related quality of life.

One trial did not provide any information on sponsorship; one trial received a national research grant, one trial by the Pedersen foundation, and three were industry-funded trials.

We found five ongoing randomised clinical trials.

Authors' conclusions

The evidence is very uncertain about the effects of gene therapy on the studied outcomes because of high risk of bias and imprecision of outcome results. The trials were underpowered and lacked trial data on clinically important outcomes. There was only one trial per comparison, and we could not perform meta-analyses. Therefore, we do not know if gene therapy may reduce, increase, or have little to no effect on all-cause mortality or overall survival, or serious adverse events in adults with unresectable hepatocellular carcinoma. The impact of gene therapy on adverse events needs to be investigated further. Evidence on the effect of gene therapy on health-related quality of life is lacking.

PLAIN LANGUAGE SUMMARY

What are the benefits and risks of gene therapy (replacing defective genes with normal genes) for treating people with operable or inoperable hepatocellular carcinoma (primary liver cancer)?

Key messages

— We found six small trials, with problems with trial design, low numbers of people taking part, and variability in results. All the people in the trials had inoperable hepatocellular carcinoma (primary liver cancer), none had operable hepatocellular carcinoma.

— We do not know whether any of the tested gene therapies when used alone, or in combination with another treatment, affects risk of death, liver function, or causes unwanted effects.

— We need well-designed, well-reported trials that focus on outcomes such as death, quality of life, and costs, which are important to people with hepatocellular carcinoma and to decision makers.

What is gene therapy?

In gene therapy, abnormal or defective genes are replaced with normal genes.

What is hepatocellular carcinoma?

Hepatocellular carcinoma is a type of primary liver cancer (i.e. a cancer that starts in the liver). People who are obese, misuse alcohol, or have chronic (long-lasting) infections with hepatitis B or hepatitis C viruses, are most at risk of developing hepatocellular carcinoma.

How is hepatocellular carcinoma treated?

The choice of treatment for people with hepatocellular carcinoma depends on how advanced their disease is. Possible treatments include chemotherapy, surgery, or liver transplantation.

What did we want to find out?

We wanted to find out if gene therapy was better than any other treatment for people with operable or inoperable hepatocellular carcinoma.

We also wanted to know if gene therapy improves liver function (how well the liver filters the blood and breaks down poisonous substances) or causes any unwanted effects, including death.

What did we do?

We identified relevant randomised trials, that is, studies in which people are assigned by chance to one of two or more treatment groups, to find out which treatment is best. We summarised the results and rated our confidence in the evidence, based on factors such as trial quality and methods.

What did we find?

We found 6 trials on a total of 364 people with advanced inoperable hepatocellular carcinoma. No trials assessed the effects of gene therapy in people with operable hepatocellular carcinoma.

All 6 trials had problems with design and conduct. The gene therapies investigated were:

— pexastimogene devacirepvec (Pexa-Vec) plus best supportive care;

— a single- or double-dose of adenovirus-thymidine kinase plus ganciclovir (ADV-TK/GCV) plus liver transplantation;

— recombinant adenovirus-p53 (rAd-p53) plus hydroxycamptothecin (an anticancer medicine);

— recombinant adenovirus human p53/5-fluorouracil (rAd-p53/5-Fu) plus transarterial chemoembolisation (injection of an anticancer medicine directly into the blood supply to the tumour); and

— E1B-deleted (dl1520) adenovirus.

The largest trial included 129 people, and the smallest trial included 10 people; 4 trials were conducted in China, 1 in Egypt, and 1 in several countries. The trials lasted from 6 months to 5 years. Trial funding came from industries, local health institutions, foundations, researchers, or the universities at which the people running the trials worked.

The trials compared gene therapy against:

— best supportive care;

— liver transplantation (where a diseased liver is replaced with a healthy one);

— transarterial chemoembolisation; or

— percutaneous ethanol (alcohol) injection directly into the tumour through the skin.

Each trial compared a different combination of treatments, so we could not combine data from all trials to obtain conclusive results. Data on clinically relevant outcomes were also missing.

Main results

We are very uncertain whether:

— Pexa-Vec plus best supportive care affects risk of death from any causes after 20 months compared to best supportive care alone;

— a single dose of ADV-TK/GCV plus liver transplantation affects risk of death from any cause after two years compared to liver transplantation alone.

— rAd-p53/5-Fu plus transarterial chemoembolisation affects disease progression (whether the cancer gets worse) compared to transarterial chemoembolisation alone;

— rAd-p53 plus hydroxycamptothecin affects disease progression compared to hydroxycamptothecin alone;

— dl1520 plus a percutaneous ethanol inection has any effect on disease progression or non-serious unwanted effects, compared to a percutaneous ethanol injection alone.

The evidence suggests that a double-dose of ADV-TK/GCV plus liver transplantation may reduce death from any cause after 5 years compared to liver transplantation alone.

What are the limitations of the evidence?

The people in the trials seemed to be aware of the treatments they received. The trials had problems with their methods and their results were likely to exaggerate or underestimate the benefits of treatment and unwanted effects. Our findings were based on only 1 trial for each gene therapy, with few data. The lack of trials and data prevents us from drawing firm conclusions.

How up to date is this evidence?

The evidence is up to date to 20 January 2023.

Summary of findings 1. Pexastimogene devacirepvec (Pexa-Vec) plus best supportive care versus best supportive care alone for unresectable hepatocellular carcinoma

Gene therapy with pexastimogene devacirepvec (Pexa-Vec) plus best supportive care versus best supportive care alone for unresectable hepatocellular carcinoma

Patient or population: participants with advanced hepatocellular carcinoma

Settings: hospital

Intervention: pexastimogene devacirepvec (Pexa-Vec) plus best supportive care

Comparison: best supportive care

Outcomes	Illustrative comparative risks* (95% CI)		Relative effect (95% CI)	No of participants and RCTs	Certainty of the evidence (GRADE)	Comments
	Assumed risk	Corresponding risk				
	Best supportive care alone	Pexa-Vec plus best supportive care				
Overall survival (absolute effect size estimates based on survival rate at 20 months) **(censored observation)**	99 per 1000	117 per 1000 (78 to 173)	**HR 1.19** (0.78 to 1.82)	129 (1 RCT)	⊕⊖⊖⊖ **Very low**[a,b]	—
Proportion of people with ≥ 1 serious adverse events (at 20 months)	140 per 1000	198 per 1000 (84 to 465)	**RR 1.42** (0.60 to 3.33)	129 (1 RCT)	⊕⊖⊖⊖ **Very low**[a,b]	The trial reported no other serious adverse events than deaths.
Health-related quality of life **Follow-up: 20 months (end of treatment)**	Assessment of quality of life and time to symptomatic progression was confounded by the high patient dropout rate	—	—	129 (1 RCT)	⊕⊖⊖⊖ **Very low**[a,c,e]	The tool used for quality of life was not mentioned.
Proportion of people with disease progression	163 per 1000	430 per 1000 (210 to 884)	**RR 2.64** (1.29 to 5.43)	129 (1 RCT)	⊕⊖⊖⊖ **Very low**[a,c]	—

Follow-up: 20 months (end of treatment)						
Adverse events considered non-serious (number of participants) Follow-up: 20 months (during treatment)	488 per 1000	977 per 1000 (718 to 1000)	**RR 2.00** (1.47 to 2.72)	129 (1 RCT)	⊕⊖⊖⊖ **Very low**[c,d]	—

*__The risk in the intervention group__ (and its 95% confidence interval) is based on the __assumed risk__ in the comparison group and the __relative effect__ of the intervention (and its 95% CI).

CI: confidence interval; **HR:** hazard ratio; **RCT:** randomised controlled trial; **RR:** risk ratio.

GRADE Working Group grades of evidence
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 effect.

[a]Downgraded two levels for serious study limitations (overall high risk of bias).
[b]Downgraded two levels for very serious imprecision (the sample size was below the 'rule of thumb' of 400 participants; wide CI crossing the line of no effect).
[c]Downgraded two levels for very serious imprecision (the sample size was below the 'rule of thumb' of 400 participants; CIs around the effect size were wide).
[d]Downgraded two levels for serious study limitation: overall high risk of bias (problems with randomisation, high rate of missing data in the control group).
[e]Narrative synthesis without providing the estimates.

Summary of findings 2. Adenovirus-thymidine kinase with ganciclovir (ADV-TK/GCV) plus liver transplantation versus liver transplantation for unresectable hepatocellular carcinoma

Adenovirus-thymidine kinase with ganciclovir (ADV-TK/GCV) plus liver transplantation versus liver transplant for unresectable hepatocellular carcinoma

Patient or population: participants with unresectable hepatocellular carcinoma

Settings: hospital

Intervention: adenovirus-thymidine kinase with ganciclovir (ADV-TK/GCV) plus liver transplantation

Comparison: liver transplantation

Outcomes	Illustrative comparative risks* (95% CI)		Relative effect (95% CI)	No of participants and RCTs	Certainty of the evidence (GRADE)	Comments
	Assumed risk	Corresponding risk				

	Liver transplantation	ADV-TK/GCV plus liver transplantation				
All-cause mortality Follow-up: 24 months	773 per 1000	301 per 1000 (155 to 587)	**RR 0.39** (0.20 to 0.76)	45 (1 RCT)	⊕⊖⊖⊖ **Very low**[a,b]	—
Proportion of people with ≥ 1 serious adverse events during treatment	—	—	—	—	—	The trial reported no serious adverse events other than mortality.
Health-related quality of life	—	—	—	—	—	No report on this outcome.
Proportion of people with disease progression	—	—	—	—	—	No report on this outcome.
Adverse events considered non-serious (number of participants) Follow-up: 24 months	0 /22 (not estimable risk) 0 per 1000	10 /23 (not estimable risk due to 0 control events) 0 per 1000 (0 to 0)	**RR 20.13** (1.25 to 324.00)	45 (1 RCT)	⊕⊖⊖⊖ **Very low**[a,c]	**ADV-TK therapy:** mild catarrhal symptoms reported in 10/23 participants; slight fever with no chills observed after injection of ADV-TK in the first 3 days in the same participants. Temperatures ranged from 37.3 °C to 38.3 °C. The same 10 participants also experienced light headaches. All these symptoms subsided in 5 days.

*__The risk in the intervention group__ (and its 95% confidence interval) is based on the __assumed risk__ in the comparison group and the __relative effect__ of the intervention (and its 95% CI).

CI: confidence interval; **RCT:** randomised controlled trial; **RR:** risk ratio.

GRADE Working Group grades of evidence
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 effect.

[a]Downgraded one level for study limitations (some concerns for risk of bias).
[b]Downgraded two levels for very serious imprecision (one small study, sample size was below the 'rule of thumb' of 400 participants; CIs around the effect size were wide).
[c]Downgraded two levels for very serious imprecision (data provided by one small study with few events; sample size was below the 'rule of thumb' of 400 participants; CIs around the effect size were wide).

Summary of findings 3. Double-dose adenovirus-thymidine kinase with ganciclovir (ADV-TK/GCV) plus liver transplantation versus liver transplantation for unresectable hepatocellular carcinoma

Double-dose adenovirus-thymidine kinase with ganciclovir (ADV-TK/GCV) plus liver transplantation versus liver transplantation for unresectable hepatocellular carcinoma

Patient or population: participants with unresectable hepatocellular carcinoma

Settings: hospital

Intervention: double dose adenovirus-thymidine kinase with ganciclovir (ADV-TK/GCV) plus liver transplantation

Comparison: liver transplantation

Outcomes	Illustrative comparative risks* (95% CI)		Relative effect (95% CI)	No of participants and RCTs	Certainty of the evidence (GRADE)	Comments
	Assumed risk	Corresponding risk				
	Liver transplantation	ADV-TK/GCV plus liver transplantation (double dose)				
All-cause mortality Follow-up: median 5 years	581 per 1000	233 per 1000 (128 to 424)	**RR 0.40** (0.22 to 0.73)	86 (1 RCT)	⊕⊕⊝⊝ **Low**[a,b]	—
Proportion of people with ≥ 1 serious adverse events during treatment Follow-up: median 5 years	—	—	—	—	—	The trial reported no serious adverse events other than mortality (see previous outcome).
Health-related quality of life	—	—	—	—	—	No report on this outcome.
Proportion of people with disease progression	—	—	—	—	—	No report on this outcome.
Adverse events considered non-serious	—	—	—	—	—	No report on this outcome.

*__The risk in the intervention group__ (and its 95% confidence interval) is based on the **assumed risk** in the comparison group and the **relative effect** of the intervention (and its 95% CI).

CI: confidence interval; **RCT:** randomised controlled trial; **RR:** risk ratio.

GRADE Working Group grades of evidence
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 effect.

[a]Downgraded one level for serious risk of bias (low number of participants).
[b]Downgraded one level for serious imprecision (data provided by one small study; sample size was below the 'rule of thumb' of 400 participants).

Summary of findings 4. Recombinant human adenovirus-p53 and hydroxycamptothecin (rAd-p53/HCT) versus hydroxycamptothecin for unresectable hepatocellular carcinoma

Recombinant human adenovirus-p53 and hydroxycamptothecin (rAd-p53/HCT) versus hydroxycamptothecin for unresectable hepatocellular carcinoma

Patient or population: participants with unresectable hepatocellular carcinoma

Settings: hospital

Intervention: recombinant human adenovirus-p53 and hydroxycamptothecin (rAd-p53/HCT)

Comparison: hydroxycamptothecin

Outcomes	Illustrative comparative risks* (95% CI)		Relative effect (95% CI)	No of participants and RCTs	Certainty of the evidence (GRADE)	Comments
	Assumed risk	Corresponding risk				
	Hydroxycamptothecin	rAd-p53/HCT				
Overall survival Censored follow-up data: at 12 months	0/18	2/30	**RR 3.06** (0.16 to 60.47)	48 (1 RCT)	⊕⊖⊖⊖ **Very low**[a,b]	—
Proportion of people with ≥ 1 serious adverse events Follow-up: maximum 2 years	—	—	—	—	—	No report on this outcome.
Health-related quality of life	—	—	—	—	—	No report on this outcome.

Proportion of people with disease progression Follow-up: 6 months	2/18	8/30	**RR 2.40** (0.57 to 10.08)	48 (1 RCT)	⊕⊖⊖⊖ **Very low**[a,b]	—
Adverse events considered non-serious (number of events) Follow-up: maximum 2 years	In 14 participants with moderate or severe ascites in the treatment group, ascites was significantly absorbed in 9 participants with the manifestations of no or mild ascites, whereas there was no such improvement in 9 participants with moderate or severe ascites in the control group.	—	—	48 (1 RCT)	⊕⊖⊖⊖ **Very low**[a,c,d]	—

*__The risk in the intervention group__ (and its 95% confidence interval) is based on the __assumed risk__ in the comparison group and the __relative effect__ of the intervention (and its 95% CI).

CI: confidence interval; **RCT:** randomised controlled trial; **RR:** risk ratio.

GRADE Working Group grades of evidence
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 effect.

[a] Downgraded one level for study limitations (some concerns for overall risk of bias).
[b] Downgraded two levels for very serious imprecision (one small study with few events; sample size was below the 'rule of thumb' of 400 participants; wide CIs including both appreciable benefit and harm).
[c] Downgraded two levels for very serious imprecision (one small study with few events; sample size was below the 'rule of thumb' of 400 participants).
[d] Narrative synthesis without providing the estimates.

Summary of findings 5. Recombinant human adenovirus-p53 and 5-fluorouracil (rAd-p53/5-Fu) plus transarterial chemoembolisation versus transarterial chemoembolisation for unresectable hepatocellular carcinoma

Recombinant human adenovirus-p53 and 5-fluorouracil (rAd-p53/5-Fu) plus transarterial chemoembolisation versus transarterial chemoembolisation for unresectable hepatocellular carcinoma

Patient or population: participants with unresectable hepatocellular carcinoma

Settings: hospital

Intervention: recombinant human adenovirus-p53 and 5-fluorouracil (rAd-p53/5-Fu) plus transarterial chemoembolisation (TACE)

Comparison: TACE

Outcomes	Illustrative comparative risks* (95% CI)		Relative effect (95% CI)	No of participants and trials	Certainty of the evidence (GRADE)	Comments
	Assumed risk	Corresponding risk				
	TACE	rAd-p53/5-Fu plus TACE				
Overall survival	—	—	—	—	—	No report on this outcome.
Proportion of people with ≥ 1 serious adverse events	—	—	—	—	—	No report on this outcome.
Health-related quality of life	—	—	—	—	—	No report on this outcome.
Proportion of people with disease progression Follow-up: median of 12.8 months	261 per 1000	224 per 1000 (88 to 564)	**RR 0.86** (0.34 to 2.16)	46 (1 RCT)	⊕⊖⊖⊖ **Very low**[a,b]	—
Adverse events considered non-serious (number of events) Follow-up: median of 12.8 months	—	—	—	—	—	No report on this outcome.

*__The risk in the intervention group__ (and its 95% confidence interval) is based on the **assumed risk** in the comparison group and the **relative effect** of the intervention (and its 95% CI).

CI: confidence interval; **RCT:** randomised controlled trial; **RR:** risk ratio.

GRADE Working Group grades of evidence
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 effect.

[a] Downgraded one level for study limitations (some concerns for overall risk of bias).
[b] Downgraded two levels for very serious imprecision (data provided by one small study; sample size was below the 'rule of thumb' of 400 participants; wide CIs crossing the line of no effect).

Summary of findings 6. E1B-deleted (dl1520) adenovirus versus percutaneous ethanol injection

Gene therapy with E1B-deleted (dl1520) adenovirus versus percutaneous ethanol injection (PEI) for unresectable hepatocellular carcinoma

Patient or population: participants with unresectable hepatocellular carcinoma

Settings: hospital

Intervention: E1B-deleted (dl1520) adenovirus

Comparison: percutaneous ethanol injection (PEI)

Outcomes	Illustrative comparative risks* (95% CI)		Relative effect (95% CI)	No of participants and RCTs	Certainty of the evidence (GRADE)	Comments
	Assumed risk	Corresponding risk				
	E1B-deleted (dl1520)	PEI				
Overall survival	—	—	—	—	—	No report on this outcome.
Proportion of people with ≥ 1 serious adverse events	—	—	—	—	—	No report on this outcome.
Health-related quality of life	—	—	—	—	—	No report on this outcome.
Proportion of people with disease progression Follow-up: 2 weeks after cessation of treatment	600 per 1000	798 per 1000 (348 to 1000)	**RR 1.33** (0.58 to 3.09)	10 (1 RCT)	⊕⊖⊖⊖ **Very low**[a,b]	—
Adverse events considered non-serious (number of participants) Follow-up: 2 weeks after cessation of treatment	1000 per 1000	820 per 1000 (490 to 1000)	**RR 0.82** (0.49 to 1.38)	10 (1 RCT)	⊕⊖⊖⊖ **Very low**[a,b]	—

*__The risk in the intervention group__ (and its 95% confidence interval) is based on the __assumed risk__ in the comparison group and the __relative effect__ of the intervention (and its 95% CI).

CI: confidence interval; **RCT:** randomised controlled trial; **RR:** risk ratio.

GRADE Working Group grades of evidence

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 effect.

---

[a]Downgraded one level for study limitations (some concerns for overall risk of bias).
[b]Downgraded two levels for very serious imprecision (data provided by one small study; sample size was below the 'rule of thumb' of 400 participants; CIs were wide and crossed the line of no effect).

BACKGROUND

Description of the condition

Hepatocellular carcinoma is the most common type of primary liver cancer that accounts for 70% to 85% of all individuals with primary liver cancer (Forner 2018; Perz 2006). Hepatocellular carcinoma is the sixth-leading cause of cancer (Asrani 2019; Fitzmaurice 2019) and the fourth-leading cause of cancer-related deaths worldwide (i.e. 810,000 in 2015) (Asrani 2019). There is a substantial diversity in age, sex, and geographic distribution, where the highest risk was reported in the East-Asia region, followed by Micronesia and Northern Africa (Bray 2018). According to the GLOBOCAN 2018 study, the age-standardised incidences were highest in Eastern Asia, followed by South-Eastern Asia and Northern Africa (Bray 2018; Petrick 2016a). Hepatocellular carcinoma is more common in men than women, which is likely the result of sex-specific behaviours that affect the risk factors for the disease (Bray 2018). For instance, the main risk factors for hepatocellular carcinoma are chronic infection with hepatitis B virus or hepatitis C virus, aflatoxin-contaminated foodstuffs, non-alcoholic steatohepatitis (NASH) (Hashimoto 2009), higher alcohol intake, obesity, smoking, type 2 diabetes (Bray 2018), and exposure to chemicals such as vinyl chloride (Uccello 2012). Studies have reported that around 40% of hepatocellular carcinomas are due to hepatitis B virus or hepatitis C virus infections, 11% to excessive alcohol consumption, and 10% to other, non-specific causes (Asrani 2019; Petrick 2018). One of the Surveillance Epidemiology End Result (SEER) registry projects predicts that the incidence of hepatocellular carcinoma will continue to rise until 2030 (Petrick 2016b).

A diagnosis of hepatocellular carcinoma is conventionally made based on findings from biopsy or imaging analyses. Treatment options for people with hepatocellular carcinoma depend on the staging and size of the tumour (Bruix 2016). The Barcelona Clinic Liver Cancer (BCLC) staging system is most commonly applied and remains the staging system recommended by the American Association for the Study of Liver Diseases (AASLD) (Singal 2023). Early-stage hepatocellular carcinoma (i.e. a single lesion of less than 5 cm or up to three nodules of less than 3 cm each) is becoming more successfully managed with different treatment modalities (hepatic resection, ablative therapy, and orthotropic liver transplantation). Management of advanced hepatocellular carcinoma remains challenging, especially for people with end-stage hepatocellular carcinoma whose lesions are usually non-resectable (Ottaviano 2017; Shi 2014). Although surgical resection is the treatment of choice for resectable hepatocellular carcinoma, it is a suitable treatment in only 10% to 35% of people with hepatocellular carcinoma (Marrero 2018; Parkin 2001).

There is evolution and adaptation of conceptual approaches to hepatocellular carcinoma management (Vitale 2023). Non-curative therapy for hepatocellular carcinoma is aimed at slowing tumour progression and prolonging survival. Potentially curative therapies such as liver transplantation and surgical resection (Marrero 2018) can only be applied to a minority of people because of advanced disease at the time of diagnosis and the lack of suitable organ donors (Hernandez-Alcoceba 2006). There are a variety of non-curative therapies, which include transarterial chemoembolisation, transarterial radioembolisation, stereotactic body radiation therapy, and systemic chemotherapy, following 'staging-guided treatment' (El-Serag 2011). Systemic therapy is currently reserved for people with unresectable hepatocellular carcinoma who are not suitable for locoregional therapy, including people with advanced-stage hepatocellular carcinoma (BCLC Stage C), some people with intermediate-stage hepatocellular carcinoma (BCLC Stage B), and those who have disease progression despite locoregional therapy (Singal 2023). Considering chemotherapeutic agents, to date sorafenib, a multiple tyrosine kinase inhibitors (mTKI) targeting the vascular endothelial growth factor (VEGF) receptor intracellular kinase pathway and other kinases, has been the only systemic chemotherapy with a proven survival benefit in hepatocellular carcinoma (Llovet 2021; Singal 2023). 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 hepatocellular carcinoma should, therefore, be balanced against the possible associated harms. Studies and systematic reviews have reported that people with hepatocellular carcinoma receive little benefit from transarterial chemoembolisation (Oliveri 2011; Perz 2006). Resistance of hepatocellular carcinoma to conventional chemotherapy and radiotherapy is associated with a high recurrence rate after radical resection. According to the SEER database, five-year survival is 21.5% (Ding 2021). Considering the limitations of the standard treatment modalities, the development of multidisciplinary therapeutic approaches to improve locoregional control and eradicate micrometastases is crucial for the improvement of survival in people with hepatocellular carcinoma.

Description of the intervention

The aim of hepatocellular carcinoma treatment is to increase survival, whilst maintaining or obtaining the highest level of quality of life. Given the complex features of tumours (hepatocellular carcinoma in this case), the molecular basis of cancer treatment such as inactivation of dominant oncogenes and activation of tumour suppressor genes has become a novel target for cancer therapy (Guo 2014; Hughes 2012). In comparison to the currently used treatment modalities, gene therapy holds a substantial potential for treatment. The European Parliament and the Council lists gene therapy as an advanced therapy under medicinal product European regulation (EC no 1394/2007) (European Parliament 2007).

Gene therapy is defined as an experimental treatment that involves introducing genetic material into a person's cells to fight or prevent disease. To deliver a gene into a cell, a carrier or vehicle (known as 'vector') is required (Micklus 2018; US National Health Library 2022). A variety of different vectors and delivery techniques have been developed (Appendix 1). Viral vectors (i.e. in vivo gene therapy) are thus far the more commonly used vectors for cancer therapy in humans. Amongst viral delivery methods, human adenovirus 5 is the most commonly used virus in gene therapy. Adenoviruses are double-stranded DNA viruses (Lee 2017). Adenoviruses and adeno-associated viruses are vectors that are used in gene delivery and can infect dividing and non-dividing cells without integrating with the genome of the host (Naso 2017). The main advantages of adenoviruses vectors are their ability to achieve a high efficiency of transduction (the transfer of genetic material from one micro-organism to another by the viral agent) (Smith 2015), high levels of gene expression (though transient), and ability to transduce non-dividing cells (Ginn 2018).

A fundamental of gene therapy is to correct the function of the abnormal gene by transferring a correct copy of the gene of interest through the use of the vehicle (i.e. gene vector) into the target organ or tissue (Delhove 2020; Dunbar 2018; Maeder 2016). The procedure of gene therapy in brief is described in Appendix 2.

Successful gene therapy often requires the long-term transgene expression provided by integrating viral vectors (Miller 2005). The introduction of new genetic material (via a vector virus or transposon (a class of genetic elements that can 'jump' to different locations within a genome)) may give rise to unpredictable outcomes such as unwanted host–vector interactions in relation to the alteration of the host genetic material (i.e. insertional mutagenesis) (Miller 2005). For example, integration can result in insertional mutagenesis and oncogene activation in two X-linked severe combined immune deficiency patients who develop leukaemia after treatment with a retroviral vector that is integrated near the LMO2 proto-oncogene (Hacein-Bey-Abina 2003).

How the intervention might work

The desirable effects of gene therapy are to deliver (tissue targeting) and activate (transcriptional targeting) a therapeutic gene to neoplastic tissue (i.e. hepatic cancer cells), without affecting healthy cells (Qian 2000).

In general, the following mechanisms of gene therapy (e.g. adenoviruses/adeno-associated virus vectors) play a potential role in hepatocellular carcinoma.

- A continuous inhibition of oncogenes, by expressing small interfering ribonucleic acid (siRNA), results in growth inhibition or induction of apoptosis (cell death) in cancer cells (Hacein-Bey-Abina 2003; Li 2005a; Li 2005b).
- An ectopic overexpression of tumour-suppressor genes (e.g. tumour protein p53 (*TP53*), cyclin-dependent kinase inhibitor 2A (*CDKN2A*)), from an adenovirus vector, results in blockade of cell division that induces apoptosis (cell death) in cancer cells (Sandig 1997).
- An intrinsic property of oncolytic activity in certain viruses (e.g. adenoviruses vector), through the cascade of eliciting antitumour immune function inhibition of tumour neovascularisation (Hacein-Bey-Abina 2003; Hermiston 2002).

More specifically, adenoviruses-mediated or adeno-associated virus serotypes for transfection of the gene product in the human body is the commonly used gene therapy option in hepatocellular carcinoma. When an adenovirus infects a normal cell, it encodes a protein (e.g. E1B), which inactivates the tumour suppressor (e.g. p53), usually acts as a checkpoint, and prevents cells from going into the S-phase (Reghupaty 2019). As such, the cell cycle will arrest, and DNA damage-repair and apoptosis, which prevent tumour progression, will occur (Dong 2014), with subsequent (tumour) cell death (Reghupaty 2019).

Why it is important to do this review

Hepatocellular carcinoma is a result of accumulation of somatic genomic alterations in passenger and driver genes, in addition to epigenetic modifications, which explains its huge molecular heterogeneity. The integration sites of viral vectors used in human gene therapy can have important consequences for efficacy and safety (Miller 2005).

Gene therapy is being assessed in order to determine whether it could be used for treatment of hepatocellular carcinoma. One non-randomised study that assessed recombinant human adenovirus type 5 plus transarterial chemoembolisation in 149 people with unresectable hepatocellular carcinoma reported a longer median overall survival time in the treatment group compared to the control group (1526 days with treatment compared to 1236 days with control; $P < 0.001$) (Dong 2014).

As gene therapy techniques are relatively new, some risks may be unpredictable (US National Health Library 2022). Although adeno-associated viruses are not known to cause disease in humans, one study of gene therapy on children with haemophilia reported that at least four out of six participants who received a higher dose of the viral vector had a transient increase in their liver enzymes suggestive of liver inflammation (Nathwani 2014). Although the characteristics of haemophilia and hepatocellular carcinoma are not identical, these findings highlight that liver inflammation is a potential concern.

In accordance with the existing guidelines in clinical practice, the use of any specific type of gene therapy should not be intended to violate the existing regulations (European Parliament 2007). The US Food and Drug Administration (FDA) regulates all gene therapy products in the USA and oversees research in this area with a focus on proof-of-principle. The US National Institutes of Health also plays an important role in ensuring the safety of gene therapy research (US National Health Library 2022). A concern, amongst others, is over the ethical context, which is whether the high costs of gene therapy could make it available only to wealthy people or nations (US National Health Library 2022). One review of four studies (three randomised clinical trials and one single-arm study), with 155 participants with primary or metastatic liver tumours (14 participants) and hepatocellular carcinoma (141 participants) reported that adverse events (mild and moderate fever, chills, headache, vomiting, and others) were decreased with repeated administration of pexastimogene devacirepvec (Pexa-Vec) (Lencioni 2015). The studies in the review were not assessed for risk of bias and the certainty of evidence was not graded. Therefore, it is difficult to determine whether or how much the published results are reliable. One systematic review including 41 studies on the acceptability of gene therapy reported that perceptions of the participants in the primary studies towards gene therapy were positive, particularly for medical reasons and fatal diseases (including cancer) but were also influenced by a perceived risk (Delhove 2020). In this context, patients themselves were integral stakeholders in the uptake of emerging genetic medicines (Delhove 2020). Therefore, we undertook a comprehensive assessment of all the available data on both the benefits and harms of gene therapy as an adjuvant treatment for hepatocellular carcinoma. We identified no systematic reviews or meta-analyses assessing the benefits and harms of gene therapy for hepatocellular carcinoma.

OBJECTIVES

To evaluate the benefits and harms of gene therapy in people with hepatocellular carcinoma, irrespective of sex, administered dose, and type of formulation.

METHODS

Criteria for considering studies for this review

Types of studies

We included randomised clinical trials with a parallel-group design that assessed gene therapy, of any type, dose, route of administration, or type of formulation, in people with hepatocellular carcinoma. Though unlikely to exist, we considered the inclusion of cluster-randomised trials and cross-over trials, if found. We did not consider the inclusion of quasi-randomised studies as their method of randomisation is not truly random; the allocation sequence generation can be anticipated by alternation, date of birth, day of admission, or medical record number (Lefebvre 2011).

We did not plan to include trials involving direct growth factor or cell therapy delivered simultaneously with gene therapy. We included trials irrespective of the language of publication, year, format, or the outcomes reported. We also aimed to include trials with unpublished data. We did not specifically search for observational studies reporting on harms, which is a limitation of the review. We are aware that by not specifically searching for all observational studies on adverse events, we introduce the risk 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 included adults aged 18 years or older of either sex.

Had we found trials evaluating people with both unresectable hepatocellular carcinoma and other malignancies, we would have extracted data only on the trial participants with unresectable hepatocellular carcinoma if separate data were available and only if the trial had used a stratified design.

Types of interventions

Experimental intervention

- Any type of gene therapy

We planned to include any type of gene therapy, irrespective of the source, dosage, frequency, or route of administration (either systemically or locally).

Gene therapy was defined as a therapeutic introduction of genetic material into a person's cells to compensate for abnormal genes or to make a beneficial protein in the recipients. We also planned to consider trial authors' definitions of gene therapy for our review.

Control intervention

- Placebo, standard care, or no gene therapy

To note, standard care may include pain management, nutrition management, symptom management, psychological support, or a recommendation of chemotherapy or surgery (Kumar 2014).

We allowed co-interventions if administered equally to the experimental and control arms of a trial (e.g. transarterial chemoembolisation).

Types of outcome measures

We used the outcome data reported at the longest follow-up for our primary analyses and main conclusions because we consider the longest follow-up time point to be the most clinically relevant time point for clinicians and patients.

We also planned to assess all outcomes, irrespective of the original study design, at six, 12, and 24 months if data were available.

Primary outcomes

- All-cause mortality (if there were no data, we planned to consider the outcome 'overall survival').
- Proportion of people with one or more serious adverse event. We use the definition of serious adverse events of the International Council for Harmonisation Guidelines (ICH-GCP 2016), that is, any event that leads to death; is life-threatening; requires inpatient hospitalisation or prolongation of existing hospitalisation; or results in persistent or significant disability, congenital birth, or anomaly; and any important medical event that may have jeopardised the patient or required intervention to prevent it. We considered all other adverse events as non-serious.
- Health-related quality of life, measured with validated questionnaires (e.g. World Health Organization Quality of Life (WHOQOL); EQ-5D, 36-item Short Form Health Survey (SF-36)).

Secondary outcomes

- Proportion of people with disease progression.
- Proportion of people with adverse events considered non-serious or not included in the definition of serious adverse events.
- Proportion of people without improvement in liver function tests (e.g. unchanged or increased activity of alanine aminotransferase or aspartate aminotransferase).

Search methods for identification of studies

Electronic searches

We searched The Cochrane Hepato-Biliary Group Controlled Trials Register, which was searched internally by the Cochrane Hepato-Biliary Group Information Specialist via the Cochrane Register of Studies Web. We also searched the Cochrane Central Register of Controlled Trials (CENTRAL) in the Cochrane Library, MEDLINE Ovid, Embase Ovid, LILACS (BIREME; Latin American and Caribbean Health Science Information database), Science Citation Index Expanded (Web of Science), and Conference Proceedings Citation Index – Science (Web of Science). The latter two were searched simultaneously through the Web of Science. The last search was on 20 January 2023.

Appendix 3 gives the search strategies for the respective databases, with the date range of the searches.

Searching other resources

We searched online trial registries such as the US National Institutes of Health Ongoing Trials Register ClinicalTrials.gov (clinicaltrials.gov/), European Medicines Agency (EMA) (www.ema.europa.eu/ema/), World Health Organization International Clinical Trials Registry Platform (WHO ICTRP) (www.who.int/ictrp), the US FDA (www.fda.gov), EU Clinical Trials

Register (www.clinicaltrialsregister.eu/), as well as pharmaceutical company sources for ongoing or unpublished trials. We also searched for grey literature in OpenGrey (www.opengrey.eu/). The last search in these online registries was 3 February 2023.

We checked the reference lists of all primary study reports and review articles for additional trials. In addition, we searched relevant manufacturers' websites for study information (e.g. Novartis (www.novartis.com/our-science/novartis-global-pipeline) and Shanghai Sunway Biotech Co, Ltd (www.sunwaybio.com.cn/PC/Content?title=ListedProducts)). The last search was 9 February 2023.

We examined papers for any relevant retraction statements and errata as errata can reveal important limitations or even fatal flaws in the included studies (Lefebvre 2022). We checked Zotero (www.zotero.org/blog/retracted-item-notifications/) and Retraction Watch Database (retractiondatabase.org/RetractionSearch.aspx).

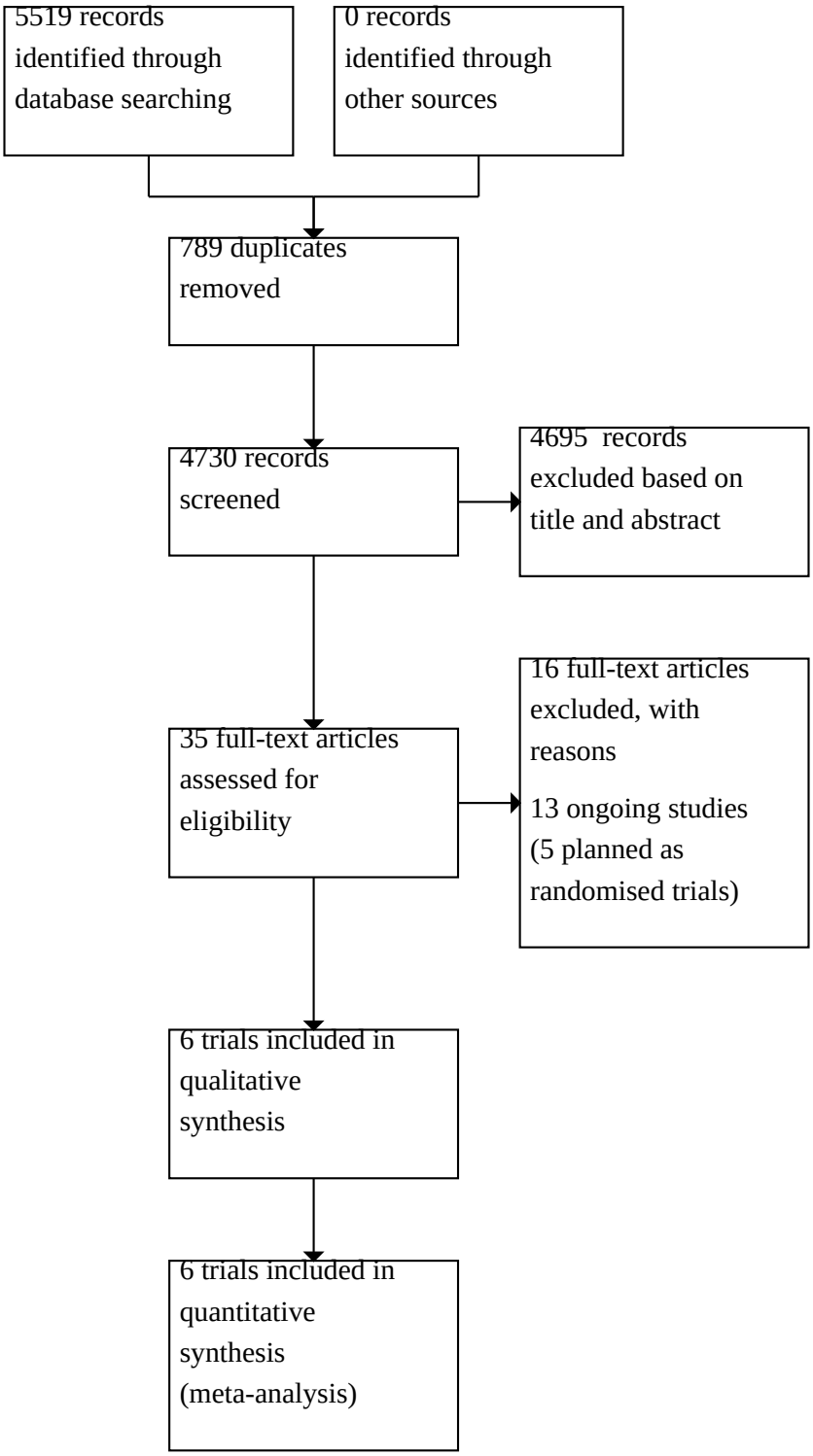
Data collection and analysis

We performed the review following the instructions in the *Cochrane Handbook for Systematic Reviews of Interventions* for data collection and analysis (Higgins 2022a).

Selection of studies

Two review authors (HHA and CN) independently screened the titles and abstracts identified by the searches and coded them as 'retrieve' (eligible or potentially eligible/unclear) or 'do not retrieve'. We retrieved full-text study reports of all potentially eligible trials, and the same two review authors independently screened the study reports for inclusion and recorded the reasons for exclusion of ineligible studies. We settled any disagreements through discussion or by consulting a third review author (HN/DK) when required. We identified and excluded duplicates and collated multiple reports of the same study so that each study, rather than each report, was the unit of interest in the review. We recorded and presented the selection process in a PRISMA flow diagram (Page 2021a; Page 2021b; Figure 1).

Figure 1. Study flow diagram (Page 2021a; Page 2021b). Date of last search 20 January 2023



Data extraction and management

Two review authors (CN and HHA) independently piloted a data collection form and extracted outcome data from included studies. One review author (CN) transferred data to Review Manager (RevMan 2020), and two review authors (DK and HN) checked that the data were entered correctly.

We extracted the following study characteristics.

- Methods: study design, study period, number of study centres and location, study setting, withdrawals/dropouts, and date of the study.
- Participants: mean age, age range, sex, diagnostic criteria, diagnostic methods, the severity of the condition, baseline liver function, smoking history, 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 the risk of bias assessment.
- Time points of the outcome data.
- Notes: funding for studies and notable conflicts of interest of trial authors.

For time-to-event data (e.g. overall survival), we aimed to extract the hazard ratios (HRs) from published data according to the technical guidance in Parmar 1998 and Tierney 2007, with corresponding measures of variance or the necessary data to calculate the HRs with 95% confidence intervals (CI). If it was not possible to estimate the HR, we planned to extract the number of participants in each arm who experienced the outcome of interest at a specific time point in order to estimate the risk ratio (RR) with 95% CI.

For dichotomous outcomes (e.g. severe adverse events), we extracted the number of participants in each arm who experienced the outcome of interest, and the number of participants assessed at the certain time point in order to estimate an RR.

For continuous outcomes (e.g. quality of life measures), we planned to extract the mean and standard deviation (SD) between the final value of the outcome measure in each trial arm at the end of follow-up. If the SDs of final values were not available, we planned to use change scores if their SDs were available. If no SDs were available, we planned to contact the corresponding authors to obtain missing data or impute these data using the methods described in Chapter 6 of the *Cochrane Handbook for Systematic Reviews of Interventions* (Higgins 2022b).

Two review authors (CN and HN) independently extracted outcome data from the included trials. We noted in the Characteristics of included studies table if outcome data were not reported in a useable way. We resolved any disagreements by consensus, involving a third review author (HHA/NHH).

One review author (CN) performed data entry into the Characteristics of included studies table in Review Manager (RevMan 2020). Another review author (HHA) checked the trial characteristics for accuracy against the trial report.

Assessment of risk of bias in included studies

Two review authors (CN and HN) independently assessed risk of bias for each study using the RoB 2 tool (www.riskofbias.info/; accessed 18 March 2022) (Higgins 2022c). We resolved any disagreements by consensus involving a third review author (HHA/NHH).

We used the intention-to-treat (ITT) principle, which includes all randomised participants, irrespective of the interventions that participants actually received.

We assessed the following five domains for each outcome in the randomised trials.

- 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
- Bias in selection of the reported result (Higgins 2022c)

For each domain, a series of signalling questions with the answers (yes, probably yes, no information, probably no, no) was used. Elaborations on these signalling questions can be found in Chapter 8 of the *Cochrane Handbook for Systematic Reviews of Interventions* (Higgins 2022c). We determined the risk of bias in each domain (low risk, some concerns, and high risk). We included text alongside the judgements to provide supporting information for our decisions (see Risk of bias in included studies).

We assigned one of the three levels of judgement to each domain, as indicated below.

- Low risk of bias: the trial was judged at low risk of bias for all domains for this result.
- Some concerns: the trial was judged to raise some concerns in at least one domain for this result, but was not at high risk of bias for any of the remaining domains.
- High risk of bias: the trial was judged at high risk of bias in at least one domain for this result, or the study was judged to have some concerns for multiple domains in a way that substantially lowered confidence in the result.

The overall risk-of-bias judgement was the same as for the individual domains such as 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 implied that the result had an overall risk of bias at least this severe.

We used the RoB 2 Microsoft Excel tool to store the data. These data are available for view in Appendix 4.

We summarised the risk of bias in the forest plots and in the text, based on ITT.

The risk of bias assessments fed into one domain of the GRADE approach for assessing the certainty of a body of evidence (Schünemann 2022).

We focused on the results of the trials that readers would find most useful. Therefore, the summary of findings tables present the following outcomes.

- Overall survival/all-cause-mortality

- People with one or more serious adverse events
- Health-related quality of life
- Disease progression
- Adverse events considered non-serious, or not included in the definition of serious adverse events

Measures of treatment effect

We reported the RR and 95% CI for dichotomous outcomes. We planned to report continuous outcomes using the mean difference (MD) and 95% CI if we could perform a meta-analysis, using data from trials that used the same tool. We planned to use a standardised mean difference (SMD) and 95% CI to report outcomes when studies used different tools to measure the same outcome. If the SMD was less than 0.40, this would indicate a small effect, while 0.40 to 0.70 would indicate a moderate effect, and greater than 0.70 would indicate a large effect (Schünemann 2022). We used the trial authors' definitions for overall survival and progression-free survival. When time-to-event outcome data were provided as dichotomous data at a fixed time point (e.g. at least 12 months), we constructed a 2 × 2 table and expressed intervention effects as RR (Higgins 2022b). Otherwise, we reported HR and its 95% CI.

Unit of analysis issues

The unit of analysis in trials with a randomised parallel-group design is the trial participant as randomised to the trial groups. The unit of analysis in cluster-randomised trials is groups of participants (e.g. schools, villages, medical practices, patients of a single doctor, or families) as randomised to the trial groups (Higgins 2022b).

For dichotomous outcomes (e.g. presence/absence of a serious adverse event), we used participants as the 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 reported rate ratios, we planned to analyse these on the basis of events rather than participants. Where a single trial reported multiple trial groups, we planned to include only the trial groups that were relevant for our comparisons. We recorded whether the trial measured adverse events as participants with any adverse events or the number of adverse events per participant. We also planned to record occasions where multiple events in a participant had been incorrectly treated as independent without taking into account the interdependence of the events. Where the number of events appeared to be equal to the number of participants, we planned to treat the events as the unit of analysis (Higgins 2022b).

If cluster-randomised trials are identified in the future, we will do "approximate analysis of cluster-randomised trials for a meta-analysis", using an effective sample size or an inflating standard error approach, as appropriate (Higgins 2023). If cross-over trials are identified in the future, we will extract data only from the end of the first period of treatment (i.e. before the cross-over) to avoid carry-over effects (Higgins 2023).

Dealing with missing data

We followed the recommendations for dealing with missing data in the *Cochrane Handbook for Systematic Reviews of Interventions* (Deeks 2022).

We contacted the corresponding authors of several included and excluded studies for further information regarding study characteristics or missing data (Dong 2014; Guan 2011; Park 2008; Penuelas 2005; Sangro 2010; Sangro 2014; Sangro 2021; Tian 2009). We also raised awareness of our concerns to editors of the journals that published the included studies.

For dichotomous outcomes, we performed analyses using the ITT principle (Deeks 2022), which includes all participants according to their original random group allocation, irrespective of compliance or follow-up. We assumed that participants who were lost to follow-up were alive and had no serious adverse events (Newel 1992). If there were missing SDs for continuous outcomes, we contacted the corresponding author to request those data. If this information was not available, we calculated SDs using case-analysis such as imputing SDs from standard errors, CIs, t values, or P values (as appropriate) that related to the differences between means in two groups, following the guidance in the *Cochrane Handbook for Systematic Reviews of Interventions* (Higgins 2022b). When there was insufficient information to calculate the SDs, we imputed them. We had planned to replace missing SDs for 'change from baseline' with those provided in other trials for the same outcome. If this approach was not applicable, assuming that correlation coefficients from the two intervention groups were similar, we planned to impute the SD of the change from baseline for the experimental intervention, following the formula described in the *Cochrane Handbook for Systematic Reviews of Interventions* (Deeks 2022).

Only one trial author sent us the requested information (Sangro 2021).

In the future, if we identify further trials, and for any reason, we cannot analyse the data using the ITT principle and cannot assess the percentage of dropouts for each included trial or other information of relevance to the analysis, we will use the trial data as available to us (available-case analysis). For sensitivity analyses, we will include missing data by considering participants as treatment failures or successes by imputing them according to the following two scenarios (Hollis 1999).

- Extreme-case analysis favouring the experimental intervention ('best-worse' case scenario): none of the dropouts/participants lost from the experimental group, but all the dropouts/participants lost from the control group experienced the outcome, including all randomised participants in the denominator.
- Extreme-case analysis favouring the control intervention ('worst-best' case scenario): all dropouts/participants lost from the experimental group, but none from the control group experienced the outcome, including all randomised participants in the denominator.

Assessment of heterogeneity

We planned to use the I^2 statistic to measure heterogeneity amongst the trials in each analysis, interpreting this as in the *Cochrane Handbook for Systematic Reviews of Interventions* (Deeks 2022).

- 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 identified substantial heterogeneity (I^2 > 50%), we planned to explore the possible causes by prespecified subgroup analyses.

Assessment of reporting biases

We planned to assess publication biases, but it could not be done due to fewer than 10 included studies (i.e. one trial per outcome and comparison) (Higgins 2022c).

Data synthesis

If a sufficient number of clinically similar trials was available, we had planned to meta-analyse their results using a random-effects model. As trials are functionally equivalent with a common effect estimate, the random-effects model is more justified than the fixed-effect model. We planned to use the fixed-effect model as a sensitivity analysis. We presented all results with 95% CIs. We entered data for analyses into Review Manager (RevMan 2020), according to the guidance provided in the *Cochrane Handbook for Systematic Reviews of Interventions* (Deeks 2022).

We planned not to conduct meta-analysis if there was considerable unexplained heterogeneity, or if trials reported outcomes differently (e.g. impossible to calculate for the same effect measure from the available statistics) as described in Chapter 12 (Table 12.1.a) of the *Cochrane Handbook for Systematic Reviews of Interventions* (McKenzie 2022). In such a setting, we planned to 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 or at some concerns of risk of bias), or statistical heterogeneity, we planned to identify the possible reasons for heterogeneity by evaluating individual trials and their subgroup characteristics. We planned to carry out the following subgroup analyses.

- Trials at low risk of bias or at low or some concern compared to trials at high risk of bias because trials at high risk of bias may overestimate beneficial intervention effects or underestimate harmful intervention effects (Higgins 2022c).
- 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 (men compared to women) because sex-specific hormones (testosterone in males and oestrogen in women) may influence the treatment outcomes.
- Stages of hepatocellular carcinoma (e.g. early stage compared to advanced stage) because advanced-stage hepatocellular carcinoma has more tumour burden (large size of tumour) than earlier stages of hepatocellular carcinoma. Gene therapy may be complicated in a tumour with a large size compared to a tumour with a small size (i.e. early stage) for control of proliferation and further progression of cancer cells because the risk of vascular invasion and dissemination increases with the diameter of a tumour (Fuks 2012).
- Pre-existing cirrhosis compared to non-cirrhotic liver because most often, hepatocellular carcinoma is progressed from pre-existing cirrhosis, whilst some hepatocellular carcinomas are not. This difference can affect the outcomes of gene therapy.

We planned to perform subgroup analyses for the following outcomes with result data at the longest follow-up if there were sufficient data.

- All-cause mortality (or overall survival)
- Serious adverse events
- Health-related quality of life
- Proportion of people with disease progression

We planned to use the formal test for subgroup interactions in Review Manager (RevMan 2020).

Sensitivity analysis

We planned to carry out the following sensitivity analyses for the primary outcomes.

- Excluding trials at overall high risk of bias.
- Analysis conducted with the fixed-effect model.
- Trial Sequential Analysis. We calculated the information size adjusted for heterogeneity (diversity, D^2) (diversity-adjusted required information size (DARIS)) between trials using the following parameters for dichotomous outcomes (Wetterslev 2009): proportion of events in the control group estimated from the included trials; anticipated intervention effect (relative risk reduction (RRR)) of 15%, alpha of 2.5%, and beta of 10% (90% power) (Jakobsen 2014; Wetterslev 2017). For continuous outcomes, we planned to use a minimal relevant difference equal to SD/2; SD of the control group; alpha of 2.5% because of three primary outcomes; and beta of 10% (90% power); and diversity of the meta-analysis. We planned to add trials to the analysis according to the year of publication and at any risk of bias. On the basis of the required information size, we planned to construct the trial sequential monitoring boundaries for benefits, harms, and futility using the O'Brien-Fleming alpha-spending and beta-spending functions. The boundaries for benefit 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 studies may be needed until the information size is reached, or until monitoring boundaries are crossed. In Trial Sequential Analysis where the cumulative Z-value does not cross the monitoring boundaries for benefit, harm, or futility, the assessment of imprecision in GRADE (see below) is downgraded by two levels if the accrued number of participants is below 50% of the DARIS, and by one level if between 50% and 100% of DARIS. We do not downgrade for imprecision if the cumulative Z-value reached or crossed benefit, harm, futility, or DARIS (TSA 2021). A more detailed description of the Trial Sequential Analysis method is available at www.ctu.dk/tsa/ (Thorlund 2017), and

Trial Sequential Analysis of our review is shown in Figure 2 and Figure 3.

Figure 2. Adenovirus-thymidine kinase with ganciclovir (ADV-TK/GCV) plus liver transplantation versus liver transplantation alone. All-cause mortality two years after randomisation (Li 2007). The diversity-adjusted required information size (DARIS) was calculated based on all-cause mortality at two years of 77% in the control group, risk ratio reduction in the ADV-TK/GCV group of 15%, alpha of 2.5%, and beta of 10% (90% power). The required information size was 763 participants. The cumulative Z-curve (blue line) crossed the trial sequential monitoring boundaries for benefit, but not for harm (red inward sloping lines), and did not enter the trial sequential monitoring area for futility (inner-wedge with red outward sloping lines). The accrued sample size (45 trial participants) was only a fraction of the DARIS of 763 participants. The 95% trial sequential analysis adjusted CI was 0.03 to 5.94.

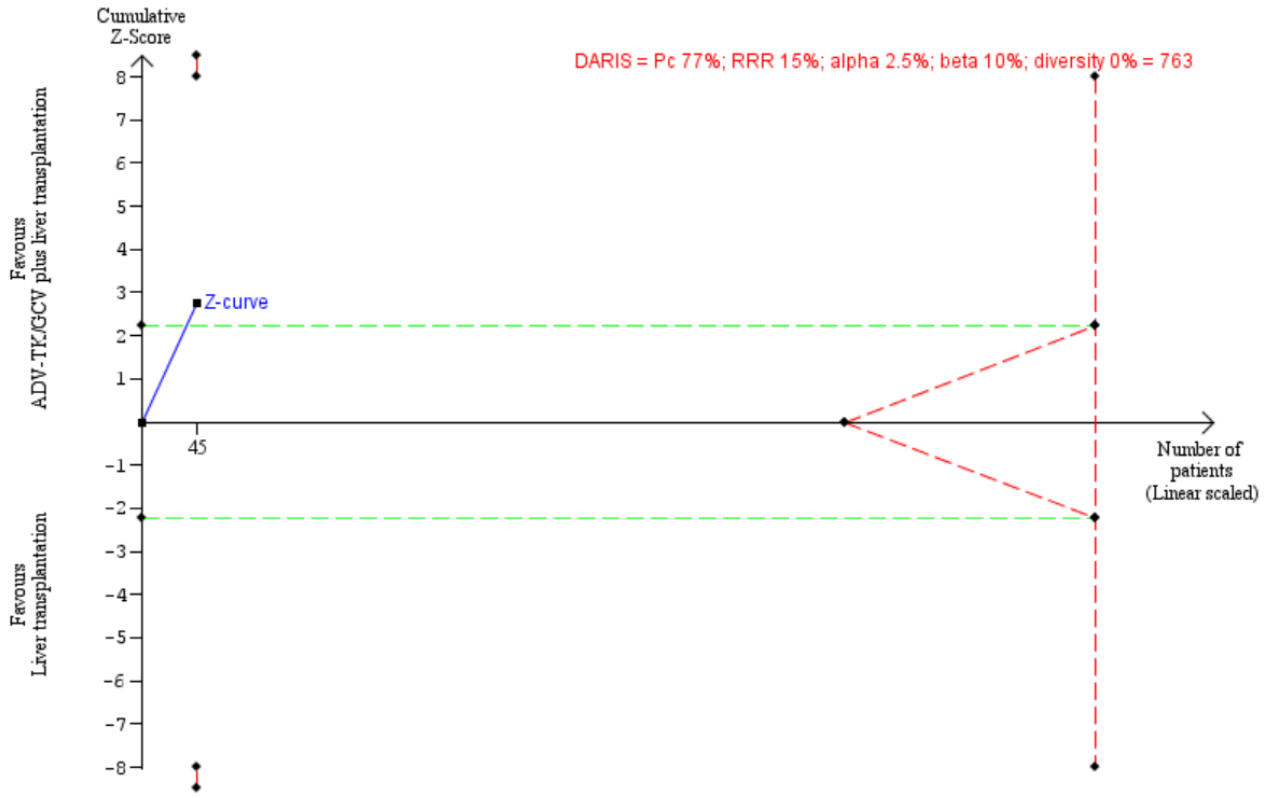
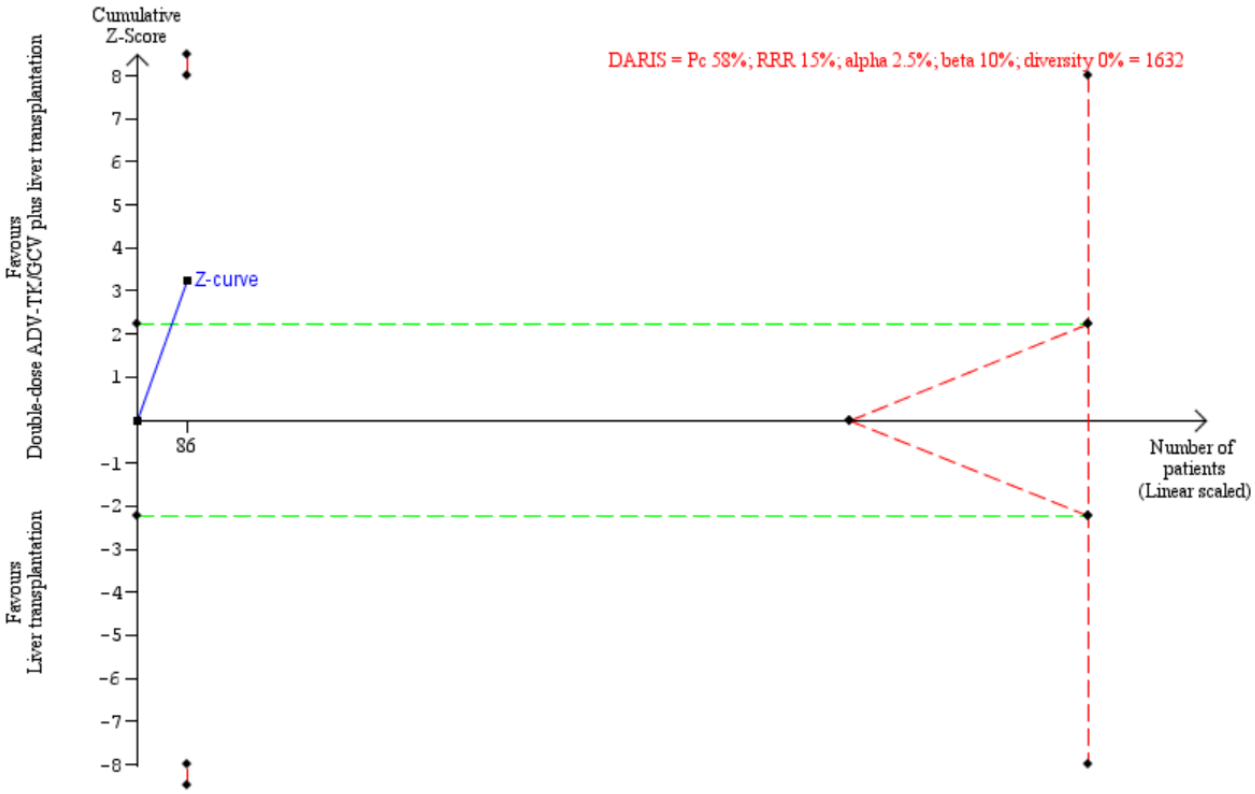


Figure 3. Double-dose of adenovirus-thymidine kinase with ganciclovir (ADV-TK/GCV) plus liver transplantation compared with liver transplantation alone All-cause mortality five years after randomisation (Zhu 2018). The diversity-adjusted required information size (DARIS) was calculated based on all-cause mortality of 58% in the control group, risk ratio reduction in the double-dose ADV-TK/GCV group of 15%, alpha of 2.5%, and beta of 10% (90% power). The required information size was 1632 participants. The cumulative Z-curve (blue line) crossed the trial sequential monitoring boundaries for benefit, but not for harm (red inward sloping lines) and did not enter the trial sequential monitoring area for futility (inner-wedge with red outward sloping lines). The accrued sample size (86 trial participants) was only a fraction of the DARIS of 1632 participants. The 95% trial sequential analysis adjusted CI was 0.04 to 3.84.



Summary of findings and assessment of the certainty of the evidence

We created six summary of findings tables on the following comparisons.

- Pexa-Vec plus best supportive care versus best supportive care alone
- Adenovirus-thymidine kinase with ganciclovir (ADV-TK/GCV) with liver transplantation versus liver transplantation alone
- Double-dose ADV-TK/GCV with liver transplantation versus liver transplantation alone
- Recombinant adenovirus human p53/hydroxycamptothecin (rAd-p53/HCT) versus hydroxycamptothecin alone
- Recombinant adenovirus human p53/5-fluorouracil (rAd-p53/5-Fu) with transarterial chemoembolisation versus transarterial chemoembolisation alone
- E1B-deleted (dl1520) adenovirus compared with percutaneous ethanol injection

We presented outcomes data analysed at the longest follow-up (Summary of findings 1; Summary of findings 2; Summary of findings 3; Summary of findings 4; Summary of findings 5; Summary of findings 6). We did not provide the mean, median, or range for the follow-up as only one trial contributed data for analysis in a comparison. However, we provided the time point for assessing the outcome.

We assessed the certainty of evidence of our predefined outcomes.

- Overall survival or all-cause mortality, depending on the data provided
- Proportion of people with one or more serious adverse events
- Health-related quality of life
- Proportion of people with disease progression
- Proportion of people with adverse events considered non-serious, or not included in the definition of serious adverse events

Two review authors (HN and CN) independently conducted GRADE assessments using GRADEpro GDT. The two review authors

resolved any discrepancy through discussion with a third author (DK) until a consensus was reached. We used the five GRADE factors (risk of bias (the overall RoB 2 judgement), heterogeneity, imprecision, indirectness, and publication bias), to assess the certainty of the body of evidence, as the certainty of evidence relates to the trials that contribute data to the meta-analysis for the prespecified outcomes. We justify all decisions to downgrade the certainty using footnotes and comments whenever needed to help the reader understand our assessments.

Regarding risk of bias, we used the overall judgement for an outcome result. Low risk of bias indicates no limitation (the certainty is not rated down); some concerns indicates either no limitation or serious limitation (the certainty is rated down one level); and high risk of bias indicates either serious limitation or very serious limitation (the certainty is rated down two levels).

We used the methods and recommendations described in Chapter 14 of the *Cochrane Handbook for Systematic Reviews of Interventions* (Schünemann 2022), using GRADEpro GDT software (GRADEpro GDT).

The levels of evidence are defined as high, moderate, low, or very low (Schünemann 2022).

- **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 effect.

We conducted the review according to our published protocol (Naing 2020). We reported any deviations from it in the Differences between protocol and review section.

RESULTS

Description of studies

See Characteristics of included studies, Characteristics of excluded studies, and Characteristics of ongoing studies tables for details.

Results of the search

We identified 5519 records from electronic database searches. We identified no additional references by handsearching the reference lists of articles retrieved through these electronic database searches. After removing 789 duplicates, we screened 4730 references. Based on title or abstract, or both, we excluded 4695 records. Amongst the remaining 35 full-text records, we excluded 16 articles. We identified 13 ongoing studies and six randomised clinical trials which met our inclusion criteria. For details of the search results, see Figure 1.

To obtain missing information needed to assess the eligibility of the trials, we contacted the corresponding authors of 10 trials (Chen 2014; Dong 2014; Guan 2011; Habib 2002; Park 2008; Penuelas 2005; Sangro 2010; Sangro 2014; Sangro 2021; Tian 2009), the principal investigator of three clinical trial protocols (NCT02395250; NCT02418988; NCT02561546), and editors of two journals (*Clinical Cancer Research* and *Anti-cancer drugs*). Only one author replied and provided the requested information (Sangro 2021).

Included studies

See Characteristics of included studies table.

We included six randomised clinical trials of parallel-group design (Chen 2014; Habib 2002; Li 2007; Moehler 2019; Tian 2009; Zhu 2018). All trials had two comparison groups. The trials were published between 2007 and 2019. Four trials were conducted in China (three single-centre (Chen 2014; Tian 2009; Zhu 2018) and one two-centre trial (Li 2007)); one in countries in North America, Asia, and Europe (a multicentre trial) (Moehler 2019), and one in Egypt (single-centre trial) (Habib 2002) (Table 1). All six trials included participants with unresectable hepatocellular carcinoma. We found no trials with participants with resectable hepatocellular carcinoma.

Participants

Overall, the trials randomly assigned 364 adults with unresectable hepatocellular carcinoma, predominantly men, with histological diagnosis of hepatocellular carcinoma.

In five trials, participants had unresectable hepatocellular carcinoma with no metastases (Chen 2014; Habib 2002; Li 2007; Tian 2009; Zhu 2018). In the remaining trial, participants had advanced hepatocellular carcinoma, radiographically confirmed progression of disease during or after sorafenib treatment, or participants were intolerant to sorafenib (Moehler 2019). Trial participants had mostly BCLC Stage C advanced (87%), had a high tumour burden in the liver, and had a median sum of longest diameters of 104 mm. These characteristics fulfil the 'not suitable for resection' criteria (Bruix 2011; Bruix 2016).

The number of participants in the six trials ranged from 10 to 129 (median 47). The median age of participants was 55.2 years, and the mean proportion of men was 72.7% (SD 20.4%) (Table 1).

Experimental interventions

We identified several distinct types of gene therapy interventions, alone or combined with another drug or intervention: Pexa-Vec (129 participants) (Moehler 2019); ADV-TK/GCV (45 participants) (Li 2007); double-dose ADV-TK/GCV (86 participants) (Zhu 2018); rAd-p53/HCT (48 participants) (Chen 2014), rAd-p53/5-Fu (46 participants) (Tian 2009), and E1B-deleted (dl1520) adenovirus (10 participants) (Habib 2002). Details of the gene therapy procedures used in the six trials are available in Appendix 2.

In five trials, participants in the experimental intervention group also received best supportive care (Moehler 2019), liver transplantation (Li 2007; Zhu 2018), hydroxycamptothecin (Chen 2014), and transarterial chemoembolisation (Tian 2009).

Control interventions

We identified the following control interventions: best supportive care (43 participants) (Moehler 2019), liver transplantation (22 participants) (Li 2007) and (43 participants) (Zhu 2018), transarterial chemoembolisation (23 participants) (Tian 2009), hydroxycamptothecin (i.e. natural anticancer drug) (18

participants) (Chen 2014), and percutaneous ethanol injection (five participants) (Habib 2002).

Best supportive care is a type of standard care (ESMO 2021). It is recommended for people with hepatocellular carcinoma with poor performance status (Eastern Cooperative Oncology Group (ECOG) performance status of 3 or 4) or Child-Pugh class C cirrhosis. It is a provision of patient care to maximise quality of life, including pain management, nutrition management, symptom management, psychological support, or a recommendation of chemotherapy or surgery (Kumar 2014).

Outcomes

Trials reported different outcomes, amongst which were all-cause mortality, health-related quality of life, disease progression, adverse events considered non-serious, and liver function test. Some trial results were only given narratively or as continuous data, such as health-related quality of life or no improvement in the liver function test. Therefore, in accordance with our protocol, we also provided these results narratively in our review.

In summary, three trials reported mortality (Li 2007; Moehler 2019; Zhu 2018) and two trials reported overall survival (Chen 2014; Moehler 2019). One trial reported health-related quality of life in a narrative format (Moehler 2019). Four trials reported disease progression (Chen 2014; Habib 2002; Moehler 2019; Tian 2009). Three trials reported adverse events considered non-serious (Habib 2002; Li 2007; Moehler 2019), while one trial reported these exclusively for the experimental group and provided no data for the control intervention group (Tian 2009) (Table 2). One trial reported no improvement in the liver function test (Habib 2002). Three trials reported this outcome in a narrative format (Chen 2014; Li 2007; Zhu 2018), and one trial did not report this outcome (Moehler 2019).

Follow-up

The range of follow-up in five trials was from two weeks to five years. One trial reported data at two weeks after end of treatment (Habib 2002); one trial reported data at censored follow-up of 20 months (Moehler 2019); one trial reported data at 12 months (Chen 2014); one trial reported data at 54 months (Li 2007); one trial reported outcome data at five years (Zhu 2018); and one trial reported data using a median 12.8 months (Tian 2009).

Dropouts

Two trials reported the number of people who dropped out (Moehler 2019; Zhu 2018). Moehler 2019 reported that all randomised participants (i.e. 86 participants in the experimental group and 43 in the control groups) were included in efficacy analyses; however, two (2.3%) participants from the experimental group and 18 participants (41.9%) from the control group were not included in the safety analysis. There was a high dropout rate in the control group (i.e. 27/43 (63%) participants were not radiographically evaluable for best response). It was stated that "therefore, no valid comparisons in response and disease control rate." Zhu 2018 reported a dropout of 4.7% (i.e. 2/43 participants) in the intervention group and 7% (i.e. 3/43 participants) in the control group.

Funding

Five trials provided information about the funding sources; industry sponsored three trials (Moehler 2019; Tian 2009; Zhu 2018), while one trial received a national research grant (Li 2007), and the Pedersen Foundation funded another trial (Habib 2002). The remaining trial did not provide any information on financial support or sponsorship (Chen 2014).

Excluded studies

We excluded 16 references during the full-text review since they failed to meet the eligibility criteria (for more information, see Characteristics of excluded studies table). Six were review articles or summary reports of studies (Hernandez-Alcoceba 2006; Jebar 2015; Lencioni 2015; Sangro 2014; Sangro 2021; Schmitz 2002); five were not randomised trials (Dong 2014, Guan 2011, and Liu 2015 were retrospective studies; Sangro 2010 had only one group; Qu 2020 used cell lines and not human participants); four were with no comparators and only within the same gene therapy intervention (Breitbach 2015; Heo 2013; Park 2008; Penuelas 2005); one was an ongoing trial with no gene therapy (NCT02309788). Liu 2015 was an observational study but as it reported adverse events, we extracted them in a narrative format.

Risk of bias in included studies

A risk of bias summary of the included trials, for each analysis, can be visualised to the right of the forest plot of each outcome. Further details on how the RoB 2 tool was applied to each domain and for each trial outcome can be found in the supplemental data files (Appendix 4).

We assessed the overall risk of bias in outcomes as at some concern in four trials (Chen 2014; Habib 2002; Li 2007; Tian 2009). The reasons for this were inadequate information on the randomisation process in three trials (Habib 2002; Li 2007; Tian 2009), and the inability to determine whether any deviations from the intended intervention arose because of the trial context and concerns over selection of the outcome results in two trials (Chen 2014; Habib 2002). The overall risk of bias was high in Moehler 2019 due to missing outcomes attributed to high dropouts and as at low risk for Zhu 2018.

We present details on the implications of assessments of risk of bias for each specific result in the Effects of interventions section.

Effects of interventions

See: **Summary of findings 1** Pexastimogene devacirepvec (Pexa-Vec) plus best supportive care versus best supportive care alone for unresectable hepatocellular carcinoma; **Summary of findings 2** Adenovirus-thymidine kinase with ganciclovir (ADV-TK/GCV) plus liver transplantation versus liver transplantation for unresectable hepatocellular carcinoma; **Summary of findings 3** Double-dose adenovirus-thymidine kinase with ganciclovir (ADV-TK/GCV) plus liver transplantation versus liver transplantation for unresectable hepatocellular carcinoma; **Summary of findings 4** Recombinant human adenovirus-p53 and hydroxycamptothecin (rAd-p53/HCT) versus hydroxycamptothecin for unresectable hepatocellular carcinoma; **Summary of findings 5** Recombinant human adenovirus-p53 and 5-fluorouracil (rAd-p53/5-Fu) plus transarterial chemoembolisation versus transarterial chemoembolisation for unresectable hepatocellular carcinoma; **Summary of findings 6** E1B-deleted (dl1520) adenovirus versus percutaneous ethanol injection

As the included trials assessed different forms of gene therapy or different dosages of the same form of gene therapy, we could not perform meta-analyses. The trials also had different control interventions (comparators).

1. Pexastimogene devacirepvec plus best supportive care versus best supportive care alone

We performed our primary analyses using outcomes data at the longest follow-up, which was 20 months (Moehler 2019). The trial did not report data at earlier time points.

1.1 Overall survival of participants at maximum follow-up (censored 20 months)

There is uncertainty about whether there may be little to no difference between the effect of Pexa-Vec plus best supportive care compared with best supportive care alone on overall survival (HR 1.19, 95% CI 0.78 to 1.82; 1 trial (censored observation at 20-month follow-up), 129 participants; very low-certainty evidence; Summary of findings 1; Analysis 1.1). The trial authors concluded that "Despite a tolerable safety profile and induction of T cell responses, Pexa-Vec did not improve overall survival as second-line therapy after sorafenib failure. The median overall survival (ITT) for Pexa-Vec plus best supportive care versus best supportive care alone was 4.2 and 4.4 months."

1.2 Serious adverse events

The trial reported no serious adverse events other than deaths. There is uncertainty about whether there may be little to no difference between the effect of Pexa-Vec plus best supportive care compared with best supportive care alone on serious adverse events (RR 1.42, 95% CI 0.60 to 3.33; 1 trial, 129 participants; very low-certainty evidence; Summary of findings 1; Analysis 1.2).

The primary reason for an adverse event-related death was hepatic failure (six participants in the Pexa-Vec plus best supportive care group (7%) and two in best supportive care group (8%)). The trial authors wrote that other reasons for an adverse event-related death were related to the worsening of liver function and progression of the disease. The treating physician stated "… while the progressive disease was the likely cause of death, the contribution of Pexa-Vec to the patient's liver failure could not be completely ruled out due to the absence of computed tomography imaging just prior to death. No deaths were considered procedure-related."

1.3 Health-related quality of life

The trial reported on quality of life only in a narrative format, stating that "assessment of quality of life and time to symptomatic progression was confounded by the high patient dropout rate." However, the trial did not mention the tool used for quality of life assessment.

1.4 Proportion of people with disease progression

There is uncertainty about whether best supportive care had less disease progression (43% of participants with Pexa-Vec plus best supportive care versus 16% of participants with best supportive care alone; RR 2.64, 95% CI 1.29 to 5.43; 1 trial, 129 participants; very low-certainty evidence; Summary of findings 1; Analysis 1.3).

1.5 Adverse events considered non-serious (number of participants)

There is uncertainty about whether best supportive care had fewer non-serious adverse events (97% of participants with Pexa-Vac plus best supportive care versus 49% of participants with best supportive care alone; RR 2.00, 95% CI 1.47 to 2.72; 1 trial, 129 participants; very low-certainty evidence; Summary of findings 1; Analysis 1.4).

The authors wrote that "… adverse events occurring more frequently amongst participants receiving Pexa-Vec were mostly mild (grade 1–2) and included pyrexia, chills, decreased appetite, nausea, hypotension, and papulopustular rash. Six participants presented with at least one adverse event related to the f intratumoural injection procedure (7%): grade 3–4. Adverse events included hypotension (2%), hepatic haemorrhage and staphylococcal sepsis, upper abdominal pain, anaemia, ascites, acute respiratory failure, fluid overload, pleural effusion, acute renal failure, and increased troponin (1% each). The overall frequency of treatment-emergent adverse events was quite high in both intervention groups, with 100% in the Pexa-Vec plus BSC [best supportive care] group and 84% in the BSC alone, group. Treatment-related grade 3 adverse events that occurred with a frequency of ≥ 5% with Pexa-Vec were pyrexia and hypotension (8% each)."

1.6 Proportion of people without improvement in liver function tests

The trial did not report on this outcome.

2. Adenovirus-thymidine kinase with ganciclovir plus liver transplantation versus liver transplantation alone

We performed our primary analyses using outcomes data at the longest follow-up, which was up to 54 months (Li 2007).

2.1 All-cause mortality at one-year follow-up

There is uncertainty about whether ADV-TK/GCV plus liver transplantation may benefit all-cause mortality at one-year follow-up (6/23 participants (13.8%) died with ADV-TK/GCV plus liver transplantation versus 13/22 (59.1%) with liver transplantation alone; RR 0.44, 95% CI 0.20 to 0.95; 1 trial, 45 participants; very low-certainty evidence; Summary of findings 1; Analysis 2.1).

2.2 All-cause mortality at two-year follow-up and until the end of the trial

There is uncertainty about whether ADV-TK/GCV plus liver transplantation may benefit all-cause mortality at two-year follow-up (7/23 participants (30.4%) died with ADV-TK/GCV plus liver transplantation versus 17/22 (77.3%) with liver transplantation alone; RR 0.39, 95% CI 0.20 to 0.76; 1 trial, 45 participants; very low-certainty evidence; Summary of findings 1; Analysis 2.2). An overall survival rate of 69.6% was maintained until the end of the trial in the ADV-TK/GCV plus liver transplantation group and an overall survival rate of 19.9% was maintained until the end of the trial in the liver transplantation group.

The median follow-up time for the ADV-TK therapy and liver transplantation group was 27.5 months (range 5 months to 50 months) and for the liver transplantation group, it was 16 months (range 2 months to 30 months).

Recurrence-free survival and overall survival

The recurrence-free survival (9.1%) and the overall survival (19.9%) in the ADV-TK therapy group plus liver transplantation were much higher than those in the liver transplantation alone group by the end of the follow-up.

2.3 Serious adverse events

The trial reported no serious adverse events other than mortality (see previous outcome).

2.4 Health-related quality of life

The trial did not report on this outcome.

2.5 Proportion of people with disease progression

The trial did not report on this outcome.

2.6 Adverse events considered non-serious (number of participants)

There is uncertainty about whether liver transplantation alone had fewer non-serious adverse events (43% participants with ADV-TK/GCV plus liver transplantation versus 0% participants with liver transplantation alone; RR 20.13, 95% CI 1.25 to 324.00; 1 trial, 45 participants; very low-certainty evidence; Summary of findings 2; Analysis 2.3). The Li 2007 publication referred the reader to supplemental material with information on safety outcomes, but we could not retrieve the supplemental material. Therefore, this result could be incomplete. See Characteristics of included studies table for further information.

2.7 Proportion of people without improvement in liver function tests

The trial reported data on serum alpha-fetoprotein levels comparing preoperation and postoperation of the two groups. Following our protocol, we did not analyse this outcome as there were no data on the proportion of participants without improvement in liver function tests.

The trial only reported that the liver function tests included serum alpha-fetoprotein, alanine aminotransferase, aspartate aminotransferase, total bilirubin, and direct bilirubin.

3. Double-dose of adenovirus-thymidine kinase with ganciclovir plus liver transplantation compared with liver transplantation alone

We performed our primary analyses using outcomes data at the longest follow-up, which was five years (Zhu 2018).

3.1 All-cause mortality at one-year follow-up

There is uncertainty about whether the double-dose ADV-TK/GCV plus liver transplantation may benefit all-cause mortality at one year (5/43 participants (11.6%) died with double-dose ADV-TK/GCV plus liver transplantation versus 14/43 (32.6%) with liver transplantation alone; RR 0.36, 95% CI 0.14 to 0.90; 1 trial, 86 participants; low-certainty evidence; Summary of findings 3; Analysis 3.1).

3.2 All-cause mortality at three-year follow-up

There is uncertainty about whether double-dose ADV-TK/GCV plus liver transplantation may benefit all-cause mortality at three years (7/43 participants (16.3%) died with double-dose ADV-TK/GCV plus liver transplantation versus 16/43 (37.2%) with liver transplantation alone; RR 0.44, 95% CI 0.20 to 0.96; 1 trial, 86 participants; low-certainty evidence; Summary of findings 3; Analysis 3.2).

3.3 All-cause mortality at five-year follow-up (data used for the main analysis)

There is uncertainty about whether double-dose ADV-TK/GCV plus liver transplantation may benefit all-cause mortality at five years (10/43 participants (23.3%) died with double-dose ADV-TK/GCV plus liver transplantation versus 25/43 (58.1%) with liver transplantation alone; RR 0.40, 95% CI 0.20 to 0.73; 1 trial, 86 participants; low-certainty evidence; Summary of findings 3; Analysis 3.3).

3.4 Serious adverse events

The trial reported no serious adverse events other than mortality (see previous outcome).

3.5 Health-related quality of life

The trial did not report on this outcome.

3.6 Proportion of people with disease progression

The trial did not report on this outcome.

3.7 Adverse events considered non-serious

The trial did not report on this outcome.

3.8 Proportion of people without improvement in liver function tests

The trial reported this outcome in a narrative format. The authors wrote "There were no significant differences in liver and renal function tests between the LT-only [lung transplantation] and LT + ADV-TK/GCV groups."

4. Recombinant human adenovirus-p53 with hydroxycamptothecin versus hydroxycamptothecin alone

We performed our primary analyses using outcomes data at the longest follow-up, which was 12 months (Chen 2014).

4.1 Overall survival at six-month follow-up

There is uncertainty about whether rAd-p53/HCT may benefit overall survival at six-month follow-up (15/30 participants (50%) were alive with rAd-p53/HCT versus 2/18 (11.1%) with hydroxycamptothecin alone; RR 4.50, 95% CI 1.16 to 17.44; 1 trial, 48 participants; very low-certainty evidence; Summary of findings 4; Analysis 4.1).

4.2 Overall survival at 12-month follow-up (data used for the main analysis)

There is uncertainty about whether there may be little to no difference between the effect of rAd-p53/HCT versus hydroxycamptothecin alone on the overall survival at 12-month follow-up (2/30 participants (6.7%) were alive with rAd-p53/HCT versus 0/18 (0%) with hydroxycamptothecin alone; RR 3.06, 95% CI 0.16 to 60.47; 1 trial, 48 participants; very low-certainty evidence; Summary of findings 4; Analysis 4.2).

The trial authors wrote that "the median survival time of patients in the treatment group was 186 days, and that of the control group was 70 days."

4.3 Serious adverse events

This outcome was not reported. However, the trial authors wrote: "In 14 patients with moderate or severe ascites in the treatment group, ascites were significantly absorbed in nine patients with the manifestations of no or mild ascites, whereas no such improvement was observed in nine patients with moderate or severe ascites in the control group."

4.4 Health-related quality of life

The trial did not report on this outcome.

4.5 Proportion of people with disease progression at six-month follow-up (data used for the main analysis)

There is uncertainty about whether there may be little to no difference between the effect of rAd-p53/HCT versus hydroxycamptothecin alone at six-month follow-up (27% participants with rAd-p53/HCT versus 11% participants with hydroxycamptothecin; RR 2.40, 95% CI 0.57 to 10.08; 1 trial, 48 participants; very low-certainty evidence; Summary of findings 4; Analysis 4.3).

4.6 Proportion of people with disease progression at 12-month follow-up

Neither of the two people alive in the rAd-p53/HCT group showed disease progression. As no one was alive in the control group, the result for disease progression was null. We did not analyse the data at the 12-month follow-up.

4.7 Adverse events considered non-serious (number of events)

We did not analyse the data. The trial authors reported that "fever to varying degrees occurred after treatment in all patients in the treatment (i.e. the experimental) group, most of them had moderate to-high-degree fever, but all were controlled. Eczema at the angles of the mouth occurred in 2 patients. Nausea, vomiting, diarrhoea, or myelosuppression were not observed in any of the patients in both the groups."

4.8 Proportion of people without improvement in liver function tests

The trial reported on this outcome but did not provide separate data for the two groups. The authors wrote "Alanine aminotransferase increased by 4-fold of the normal value in 26 patients. AFP level was significantly increased in 28 patients (> 400 ng/mL)."

5. Recombinant human adenovirus-p53 (classic tumour suppressor gene)/5-fluorouracil plus transarterial chemoembolisation versus transarterial chemoembolisation alone

We performed our primary analyses using outcomes data at the longest follow-up, which was a median 12.8 months (Tian 2009).

5.1 Overall survival

We had insufficient data to assess this outcome. It was only reported that "There were no statistically significant differences noted in time to progression (log-rank P = 0.62, Fig. 1) and overall survival (log-rank P = 0.87, Fig. 2) between the two groups" (p.391).

5.2 Serious adverse events

The trial did not report on this outcome.

5.3 Health-related quality of life

The trial did not report on this outcome.

5.4 Proportion of people with disease progression

There is uncertainty about whether there may be little to no difference between the effect of rAd-p53/5-Fu plus transarterial chemoembolisation versus transarterial chemoembolisation alone on disease progression at median follow-up of 12.8 months (26% participants with rAd-p53/5-Fu plus transarterial chemoembolisation versus 30% participants with transarterial chemoembolisation alone; RR 0.86, 95% CI 0.34 to 2.16; 1 trial, 46 participants; very low-certainty evidence; Summary of findings 5; Analysis 5.1).

5.5 Adverse events considered non-serious (number of events)

Tian 2009 (46 participants) reported there was no increase in adverse events with multiple injections of rAd-p53/5-Fu. There were no data for the two separate groups available, and we contacted the authors. The trialists reported in the article that "there were no statistically significant differences in complications of TACE (usual care) between the two groups" (Table 2). The median follow-up was 12.8 months.

5.6 Proportion of people without improvement in liver function tests

Data for the two groups were not provided separately, and we could not analyse this outcome. The authors wrote "Assessment of LFTs [liver function tests] revealed no significant rise in liver enzymes or marked effect on LFTs (prothrombin time, bilirubin, albumin) in patients receiving Ad-p53/5-Fu. Three patients had a mild rise in aspartate aminotransferase level and three patients had a minimal rise in bilirubin."

6. E1B-deleted (dl1520) adenovirus versus percutaneous ethanol injection

We performed our primary analyses using outcomes data at the longest follow-up, which was two weeks after cessation of treatment (Habib 2002).

6.1 Overall survival

The trial did not report on this outcome.

6.2 Serious adverse events

The trial did not report on this outcome.

6.3 Health-related quality of life

The trial did not report on this outcome.

6.4 Proportion of people with disease progression

There is uncertainty about whether there may be little to no difference between the effect of dl1520 adenovirus versus percutaneous ethanol injection on disease progression at two weeks after cessation of treatment (RR 1.33, 95% CI 0.58 to 3.09;

1 trial, 10 participants; very low-certainty evidence; Summary of findings 6; Analysis 6.1).

6.5 Adverse events considered non-serious (number of participants)

There is uncertainty about whether there may be little to no difference between the effect of dl1520 adenovirus versus percutaneous ethanol injection on adverse events at two weeks after cessation of treatment (RR 0.82, 95% CI 0.49 to 1.38; 1 trial, 10 participants; very low-certainty evidence; Summary of findings 6; Analysis 6.2).

6.6 Proportion of people without improvement in liver function tests

There is uncertainty about whether there may be little to no difference between the effect of dl1520 adenovirus versus percutaneous ethanol injection on number of participants without improvement in liver functions test at two weeks after cessation of treatment (RR 1.22, 95% CI 0.73 to 2.06; 1 trial, 10 participants; very low-certainty evidence; Summary of findings 6; Analysis 6.3).

GRADE judgement for all outcomes and comparisons

We assessed the certainty of evidence as very low because of risk of bias and imprecision in five out of the six comparisons (Summary of findings 1; Summary of findings 2; Summary of findings 4; Summary of findings 5; Summary of findings 6). Very low-certainty evidence means that, irrespective of the effect estimate, the true effect can be substantially different from it. We assessed the certainty of evidence as low because of imprecision in one trial (Summary of findings 3). Low-certainty of evidence means that the true effect may be substantially different from the estimate of the effect. All trials were underpowered.

Subgroup analyses

We could not perform any of the planned subgroup analyses or assess heterogeneity because there was only one trial per comparison.

Sensitivity analysis for imprecision

Although there was only one trial per comparison, we performed sensitivity analyses with Trial Sequential Analysis to assess imprecision.

Trial Sequential Analysis

1. Pexastimogene devacirepvec plus best supportive care versus best supportive care alone

- **All-cause mortality at 20 months (end of treatment)**

Only one trial provided data (Moehler 2019). The boundary of the DARIS was ignored because of too little information. Figure not shown.

- **Overall survival at 20 months (censored observation)**

Only one trial provided data (Moehler 2019). There was insufficient data to estimate the DARIS.

2. Adenovirus-thymidine kinase with ganciclovir plus liver transplantation versus liver transplantation alone

- **All-cause mortality at one-year follow-up**

Only one trial provided data (Li 2007). The boundary of the DARIS was ignored because of too little information. Figure not shown.

- **All-cause mortality at two-year follow-up**

Only one trial provided data (Li 2007). The DARIS was calculated based on all-cause mortality at two years of 77% in the control group; RRR in the ADV-TK/GCV group of 15%; alpha of 2.5%, as we used three primary outcomes; 90% power; and trial diversity was 0%. The required information size was 763 participants. The cumulative Z-curve (blue line) did cross the trial sequential monitoring boundaries for benefit, but not the harm (red inward sloping lines) and did not enter the trial sequential monitoring area for futility (inner-wedge with red outward sloping lines). The accrued sample size (45 trial participants) was only a fraction of the DARIS (763 participants). The 95% trial sequential analysis adjusted CI was 0.03 to 5.94 (Figure 2).

3. Double-dose of adenovirus-thymidine kinase with ganciclovir plus liver transplantation compared with liver transplantation alone

- **All-cause mortality at one-year follow-up**

Only one trial provided data (Zhu 2018). The boundary of the DARIS was ignored because of too little information. Figure not shown.

- **All-cause mortality at three-year follow-up**

All-cause mortality at three-years after randomisation. Only one trial provided data (Zhu 2018). The boundary of the DARIS was ignored because of too little information. Figure not shown.

- **All-cause mortality at five-year follow-up**

Only one trial provided data (Zhu 2018). The DARIS was calculated based on all-cause mortality of 58% in the control group; RRR in the double-dose ADV-TK/GCV group of 15%; alpha of 2.5%, as we used three primary outcomes; and beta of 10% (90% power). Trial diversity was 0%. The required information size was 1632 participants. The cumulative Z-curve (blue line) did cross the trial sequential monitoring boundaries for benefit, but not for harm (red inward sloping lines) and did not enter the trial sequential monitoring area for futility (inner-wedge with red outward sloping lines). The accrued sample size (86 trial participants) was only a fraction of the DARIS (1632 participants). The 95% trial sequential analysis adjusted CI was 0.04 to 3.84 (Figure 3).

4. Recombinant human adenovirus-p53 with hydroxycamptothecin versus hydroxycamptothecin alone

- **Overall survival at six-month follow-up**

Only one trial provided data (Chen 2014). The boundary of the DARIS was ignored because of too little information. Figure not shown.

- **Overall survival at 12-month follow-up**

Only one trial provided data (Chen 2014). The boundary of the DARIS could not be calculated because of zero events in the control group.

5. Recombinant human adenovirus-p53 (classic tumour suppressor gene)/5-fluorouracil plus transarterial

chemoembolisation versus transarterial chemoembolisation alone

There was only one trial for this comparison (Tian 2009). We could not plot Trial Sequential Analysis because the trial did not provide data on our primary outcomes.

6. E1B-deleted (dl1520) adenovirus compared with percutaneous ethanol injection

There was only one trial for this comparison (Habib 2002). We could not plot Trial Sequential Analysis because the trial did not provide data on our primary outcomes.

Reporting bias

We could not assess reporting bias by creating a funnel plot for any of the comparisons because there was only one trial per comparison.

DISCUSSION

Summary of main results

We included six randomised clinical trials involving 364 adults with advanced hepatocellular carcinoma who received gene therapy. The gene therapies administered in the six trials were Pexa-Vec, ADV-TK/GCV, double-dose ADV-TK/GCV, rAd-p53, and E1B-deleted (dl1520) adenovirus. Trial participants in five trials had unresectable hepatocellular carcinoma with no metastases, which was mostly confirmed histopathologically. The remaining trial included people with advanced hepatocellular carcinoma, who failed to respond to sorafenib treatment (Moehler 2019). Five trials compared one type of gene therapy intervention provided together with the same intervention as in the control group. The experimental group in Habib 2002 received only the gene therapy intervention. The comparisons in the six trials were as follows: usual care (i.e. best supportive care) (Moehler 2019), liver transplantation (Li 2007; Zhu 2018), transarterial chemoembolisation (Tian 2009), hydroxycamptothecin (Chen 2014), or percutaneous ethanol injection (PEI) (Habib 2002). Four trials were conducted in China (Li 2007; Chen 2014; Tian 2009; Zhu 2018); one in countries in North America, Asia, and Europe (Moehler 2019); and one in Egypt (Habib 2002). One trial did not provide any information on clinical trial support or sponsorship. Three trials were funded by industry, one trial by a national research grant, and one trial by an American foundation.

Outcomes

The division of outcomes into 'primary' and 'secondary' outcome measures can be helpful as it sets the standards for evaluation of interventions (Keus 2010). Only four trials studied all primary and secondary outcomes of this review, in which all-cause mortality or overall survival was a common primary outcome, followed by serious adverse events. Amongst the six trials, only three assessed a common secondary outcome, such as disease progression or adverse events considered non-serious. None of the trials provided data for analysis on health-related quality of life. However, one of the trials reported health-related quality of life in a narrative format, without providing the tool for rating the scale.

Pexa-Vec therapy combined with best supportive care versus best supportive care alone suggested no difference in effect on all-cause mortality and overall survival at 20 months after treatment. However, best supportive care alone suggested a beneficial effect on the number of people with disease progression and with any non-serious adverse events (number of participants). All these outcomes were graded as very low-certainty evidence.

When compared with liver transplantation, the standard dose of ADV-TK/GCV plus liver transplantation was found to have lower mortality at two-year follow-up and the same was noticed with double-dose ADV-TK/GCV at five-year follow-up. However, there were more adverse events with standard-dose gene therapy compared to liver transplantation alone. Surprisingly, there was lower mortality with double-dose gene therapy. Nonetheless, we are not confident in these findings as all were graded as very low- or low-certainty evidence.

rAd-p53/HCT suggested better overall survival than hydroxycamptothecin alone at six-month follow-up, but there was no difference on overall survival at 12-month follow-up and on disease progression at six-month follow-up. Similarly, rAd-p53/5-Fu plus transarterial chemoembolisation versus transarterial chemoembolisation alone suggested no difference in disease progression at median follow-up of 12.8 months. E1B-deleted (dl1520) adenovirus versus percutaneous ethanol injection also suggested no difference in disease progression and in improvement of liver function tests at two weeks after cessation of treatment. We are not confident in these findings as all were graded as very low-certainty evidence.

Similar to Pexa-Vec therapy, the standard dose of ADV-TK/GCV plus liver transplantation demonstrated more non-serious adverse events (number of events) compared with liver transplantation alone. E1B-deleted (dl1520) adenovirus reported no difference in adverse events (number of events). We are not confident in these findings as all trials were graded as very low-certainty evidence.

The results of this systematic review should be interpreted with great caution because only one of the six trials was at low risk of bias. The remaining five trials (80%) were assessed at high risk of bias or with some concerns of bias due to trial design and imprecision of results. The outcome data were subject to the risk of both type I (alpha) and type II errors (beta). Therefore, there is a risk of random error ('play of chance'), which is the risk of drawing false conclusions based on sparse data (Keus 2010).

One observational study, found during the searches for randomised clinical trials, involving 15 participants with hepatocellular carcinoma, which was not included in the analyses of our review, reported adverse events attributed to rAD-p53 gene therapy (Liu 2015).

Summary of ongoing studies

At first, we found 13 ongoing studies aiming to assess gene therapy on hepatocellular carcinoma (NCT00003147; NCT00300521; NCT00451022; NCT01071941; NCT02309788; NCT02395250; NCT02418988; NCT02509169; NCT02561546; NCT02905188; NCT03313596; NCT03680560; NCT04715191).

Two of these have now been terminated (NCT00003147; NCT03680560). We were unable to reach the trial investigators and, therefore, we cannot provide the reasons for their termination. Of the remaining 11 studies, four are prospective single-arm studies (NCT02905188; NCT02395250; NCT04715191; NCT01869088), and one is planned as a retrospective

observational study (NCT00451022). Five of the remaining six studies are planned as randomised clinical trials and for one, it was not specified. The planned randomised trials aim to investigate the following types of gene therapy: ADV-TK (NCT00300521; NCT03313596); rAd (NCT02418988; NCT02561546); and p53 (NCT02509169).

The comparators in the five trials are liver transplantation (NCT00300521; NCT03313596); transarterial chemoembolisation (NCT02418988; NCT02509169); and transcatheter embolisation (NCT02561546).

Gene therapy is an evolving field, and it assures new trials will be available as time goes on. Trials with larger sample sizes and longer follow-up times are needed to show the beneficial and harmful effects of any gene therapy type for people with unresectable hepatocellular carcinoma.

Overall completeness and applicability of evidence

Several issues need to be considered for the applicability of the findings of this review to clinical practice.

The generalisability of the findings of this review is limited by the small number of trials identified with a small quantity of data. Moreover, most of the included trials are early phase studies (i.e. phase 1 or phase 2 cancer trials). For instance, the trial with the shortest duration (i.e. Habib 2002) was a phase 2 trial. Although some of the trials suggested promising results, phase 3 trials may demonstrate different findings. Perhaps the biggest challenge to successful oncolytic virotherapy in hepatocellular carcinoma is the ability to infect an adequate number of malignant hepatocytes with a sufficiently high multiplicity of infection and to maintain viral propagation (Jebar 2015). Four trials were conducted in China, an upper- to middle-income country, which may limit the applicability of the findings to low-income countries.

In this review, there were no clinically important differences in serious adverse events between any type of gene therapy and the comparators, such as best supportive care, liver transplantation, and percutaneous ethanol injection. However, these findings were based on analyses that involved only one small trial per comparison, which is a limitation of the evidence.

The included trials did not report analysis of the impact on quality of life, which could be the most desirable benefit for people after receiving the targeted gene treatment. Only one trial provided narrative reporting but omitted information about the tool used to measure the quality of life (Moehler 2019).

Data from the identified ongoing randomised trials could contribute to the overall effect estimates of the interventions, which might alter the results of this review. We plan to update the review accordingly.

Data included in this systematic review were from six randomised clinical trials, in which every single trial compared the use of individual and specific gene therapy versus usual care, liver transplantation, hydroxycamptothecin, transarterial chemoembolisation, or percutaneous ethanol injection for adults with unresectable (five trials) and advanced (one trial) hepatocellular carcinoma. In the trials with trial results showing a beneficial difference between the experimental genetic intervention versus the control intervention, it is difficult or impossible to ascribe betterment to the experimental intervention as it could also be achieved due to a detrimental effect of the control intervention.

In summary, the applicability of the evidence of this review to current practice in people with unresectable hepatocellular carcinoma is extremely limited, and it is too premature to generalise the findings of the review. All trial results should be interpreted with caution.

Quality of the evidence

We used the GRADE criteria to assess the certainty of the evidence (Summary of findings 1; Summary of findings 2; Summary of findings 3; Summary of findings 4; Summary of findings 5; Summary of findings 6).

The GRADE assessment showed very low-certainty evidence for five of the six comparisons and low-certainty evidence for one of the comparisons. This means that any estimate of the effect is uncertain. There are two main reasons for risk of bias, which have led to downgrading the certainty of evidence. First, five trials did not clearly describe randomisation or allocation concealment (Chen 2014; Habib 2002; Li 2007; Moehler 2019; Tian 2009). Second, three trials did not explicitly report blinding of participants and personnel (Li 2007; Habib 2002; Tian 2009). We cannot say if this has influenced the reported outcomes though they are objective in nature (i.e. overall survival, disease progression) (Savovic 2018). Despite our requests to the authors and the editors of the journals published, we did not receive additional data to change judgements in these key areas. It is also worth noting that some other key sources of bias may exist, such as selective reporting, as the protocols of four trials were not available (Chen 2014; Habib 2002; Li 2007; Tian 2009), and bias due to incomplete outcome data because of a high number of dropouts (Moehler 2019). In addition, imprecision due to the small number of participants with few events as well as wide CIs in analyses reduced our confidence in the certainty of evidence of this review.

Based on the findings of this review, gene therapy may reduce or increase or have little to no effect on outcomes, but the evidence so far is very uncertain. Due to its high risk of bias, or some concerns, the results of these individual trials need to be interpreted with caution.

Potential biases in the review process

Potential biases for this review were limited due to comprehensive searches with no limit on publication status or language, with adherence to Cochrane methodology. Nonetheless, there are areas of concern in this review.

We did not specifically search for observational studies to include in this review, which may bias findings in favour of benefits of the interventions while downplaying harms, and overlooking uncommon and late adverse events (Storebø 2018). Lack of reply from the primary authors upon request for missing information or more details of participant recruitment may have resulted in 'reporting bias'. Trials reporting on outcomes in our summary of findings were evaluated with the Cochrane RoB 2 tool, which included rating of some concerns on some domains due to insufficient information. Trials were excluded due to insufficient information, and we did not receive responses from the authors or the respective journal editors (see details in Results of the search).

To identify fraudulent studies, it has been advised to contact authors of journals for more information (Lisa 2022; Liu 2022).

Agreements and disagreements with other studies or reviews

This is the first Cochrane review on gene therapy for people with unresectable hepatocellular carcinoma.

The European Parliament and the Council refer to gene therapy as an advanced therapy under medicinal product European regulation (EC No 1394/2007) (European Parliament 2007). The National Institute for Health and Care Excellence (NICE) published final draft guidance on 4 June 2021 that recommends a new and potentially curative one-off gene therapy, onasemnogene abeparvovec, for treating spinal muscular atrophy (NICE 2021). To date, we are not aware of guidelines that include recommendations for gene therapy in people with hepatocellular carcinoma. One review of four trials that included participants with liver tumours suggested that Pexa-Vec was well-tolerated with few incidences of procedure-related adverse events, including one procedure-related bleeding and sepsis event (Lencioni 2015). Although procedure-related haemorrhage and sepsis are of concern, we were unable to determine whether or to what extent the published results were reliable because we did not evaluate them methodologically.

In our review, viral vectors such as adenoviruses are commonly used options for gene therapy in people with hepatocellular carcinoma (Chen 2014; Habib 2002; Tian 2009; Zhu 2018). Regarding the efficiency of gene therapy vectors, one phase 1 trial highlighted the concerns over gene therapy vectors (i.e. first-generation adenoviruses) used to treat liver cancer (Penuelas 2005). On the favourable side, these vectors possess the ability to transduce hepatocellular carcinoma tumours with high efficiency, leaving intact the non-tumoural cirrhotic tissue when given by intratumoural injection. On the downside, they can be administered only once because they fail to reinfect the tumour after a second administration. Hence, it is critical for the assessment of new vectors for gene therapy.

One narrative review addressed two issues on which genes should be targeted, and which delivery systems should be used (Reghupaty 2019). The authors emphasised the role of non-dividing hepatocyte-infecting lentiviruses and adenoviral vectors as efficient systems for gene therapy. We were unable to support or refute their claim due to a lack of data and adequate information.

From the findings of this review, it is difficult to demonstrate evidence to support any gene therapy. That is not to suggest gene therapy is inappropriate, merely because there is still a paucity of data from randomised clinical trials to guide this key area in treatment of hepatocellular carcinoma.

AUTHORS' CONCLUSIONS

Implications for practice

Based on very low- and low-certainty evidence of this review, we still do not know if gene therapy may reduce, increase, or have little to no effect on all-cause mortality or overall survival, or serious adverse events, disease progression, and non-serious adverse events in adults with hepatocellular carcinoma. Evidence on the effect of gene therapy on health-related quality of life is lacking. Our review shows that the impact of gene therapy on adverse events needs to be further investigated, and this information should be used to inform patients considering gene therapy. Our conclusions are based on trials at mainly some concerns for risk of bias and at high risk of bias. The trials were underpowered and lacked trial data on clinically important outcomes. There was only one trial per comparison and meta-analyses were not possible to perform. We would also like to reiterate that viral vectors should be used with caution in humans due to potential adverse effects (Olowoyeye 2020).

Overall, the evidence in this review is highly insufficient, and each result should be interpreted with caution. We lack well-designed, large trials, assessing clinically and patient-relevant outcomes to demonstrate the effects of gene therapy in unresectable hepatocellular carcinoma.

Implications for research

Much more evidence from trials at low risk of bias and sufficient power is needed to conclude on a decision for or against an intervention with gene therapy in people with unresectable hepatocellular carcinoma.

Further research is indicated, including multicentre trials in larger populations with hepatocellular carcinoma using robust methods, which should be reported transparently. Trialists should define and categorise participants according to the stages of hepatocellular carcinoma and, if possible, provide disaggregated data for different subgroups. We recommend including various stages of hepatocellular carcinoma to delineate the possible impact of prior therapy or metastatic status on the evidence. Such trials should use stratified central randomisation. Further trials assessing the effect of gene therapy with participant-related outcomes (e.g. length of hospital stay, quality of life) as primary outcomes rather than liver function improvement would be more appropriate for applicability to clinical practice. Follow-up data would be beneficial, especially to identify any impact of gene therapy on recurrent hepatocellular carcinoma and readmissions. The selection of vectors as well as genetic components should be evidence based. The selection of which standard treatment should be the control therapy should also be evidence based. Once an appropriate control treatment has been identified, this should likely be offered to participants in both the experimental gene therapy intervention and in the control group. The latter group should also receive placebo to blind the trial. Due to the potential of adverse effects of using viruses as vectors in gene therapy, non-viral vector gene therapy deserves further development and testing. Therefore, the inclusion of various forms of gene therapy in various stages of hepatocellular carcinoma to delineate the possible impact of gene therapy on the evidence would be useful. Since there is no study on cost-effectiveness, future research on this outcome may also be required. Future trials should be designed according to the SPIRIT (Standard Protocol Items: Recommendations for Interventional Trials) statement (www.spirit-statement.org/) and reported according to the CONSORT statement (www.consort-statement.org/).

ACKNOWLEDGEMENTS

Cochrane Review Group funding acknowledgement: the Danish State is the largest single funder of the Cochrane Hepato-Biliary Group through its investment in the Copenhagen Trial Unit, Centre

for Clinical Intervention Research, the Capital Region of Denmark, Rigshospitalet, Copenhagen, Denmark.

Disclaimer: the views and opinions expressed in this review are those of the authors and do not necessarily reflect those of the Danish State or the Copenhagen Trial Unit.

The authors thank Marija Barbateskovic (Cochrane review author from Denmark) for comments on the review and Trial Sequential Analysis, and Kerry Dwan, UK, for her assistance in assessing risk of bias.

Editorial and peer-reviewer contributions

Cochrane Hepato-Biliary supported the authors in the development of this review.

- Sign-off Editor: Jian Ping Liu, China
- Contact Editor: Christian Gluud, Co-ordinating Editor, Denmark
- Information Specialist (developing search strategies and trial search): Sarah Louise Klingenberg, Information Specialist, Denmark
- Peer reviewer (provided comments on Trial Sequential Analyses text and figures): Mark Aninakwah Asante, Denmark.

Cochrane Central Editorial Service team supported the authors in the editorial process of this review.

The following people conducted the editorial process for this article.

- Sign-off Editor (final editorial decision): Jacob Rosenberg, Professor, Chief Surgeon, Department of Surgery, University of Copenhagen; Denmark
- Managing Editor (selected peer reviewers, provided comments and editorial guidance to authors, edited the article): Lara Kahale and Colleen Ovelman, Central Editorial Service; UK
- Editorial Assistant (conducted editorial policy checks, collated peer-reviewer comments and supported the editorial team): Sara Hales-Brittain, Central Editorial Service; UK
- Copy Editor (copy editing and production): Anne Lawson, Cochrane Central Production Service
- Peer-reviewers (provided comments and recommended an editorial decision): Associate Professor Hao-Wen Sim – NHMRC Clinical Trials Centre, University of Sydney, Sydney, Australia; Faculty of Medicine and Health, University of New South Wales, Sydney, Australia; Department of Medical Oncology, The Kinghorn Cancer Centre, Sydney, Australia; Department of Medical Oncology, Chris O'Brien Lifehouse, Sydney, Australia (clinical/content review); Aditi Bauskar, Independent Consultant (consumer review); Nuala Livingstone, Cochrane Evidence Production and Methods Directorate (methods review); Jo Platt, Central Editorial Information Specialist (search review); Simran Ochani, MD (clinical/content review).

REFERENCES

References to studies included in this review

Chen 2014 *{published data only}*

Chen S, Chen J, Xi W, Xu W, Yin G. Clinical therapeutic effect and biological monitoring of p53 gene in advanced hepatocellular carcinoma. *American Journal of Clinical Oncology* 2014;**37**:24-9. [DOI: 10.1097/COC.0b013e3181fe4688]

Habib 2002 *{published data only}*

Habib N, Salama H, Abd El Latif Abu Median A, Isac Anis I, Abd Al Aziz RA, Sarraf C, et al. Clinical trial of E1B-deleted adenovirus (dl1520) gene therapy for hepatocellular carcinoma. *Cancer Gene Therapy* 2002;**9**:254-9. [DOI: 10.1038/sj.cgt.7700431]

Li 2007 *{published data only}*

Li N, Zhou J, Weng D, Zhang C, Li L, Wang B, et al. Adjuvant adenovirus-mediated delivery of herpes simplex virus thymidine kinase administration improves outcome of liver transplantation in patients with advanced hepatocellular carcinoma. *Clinical Cancer Research* 2007;**13**:5847-54. [DOI: 10.1158/1078-0432.CCR-07-0499]

Moehler 2019 *{published data only}*

* Moehler M, Heo J, Lee HC, Tak WY, Chao Y, Paik SW, et al. Vaccinia-based oncolytic immunotherapy pexastimogene devacirepvec in patients with advanced hepatocellular carcinoma after sorafenib failure: a randomized multicenter phase IIb trial (TRAVERSE). *Oncoimmunology* 2019;**8**(8):1615817.

NCT01387555. A phase 2b study of modified vaccinia virus to treat patients advanced liver cancer who failed sorafenib (TRAVERSE). clinicaltrials.gov/ct2/show/NCT01387555 (first received 4 July 2011).

Tian 2009 *{published data only}*

Tian G, Liu J, Zhou JS, Chen W. Multiple hepatic arterial injections of recombinant adenovirus p53 and 5-fluorouracil after transcatheter arterial chemoembolization for unresectable hepatocellular carcinoma: a pilot phase II trial. *Anticancer Drugs* 2009;**20**(5):389-95. [DOI: 10.1097/CAD.0b013e32832a2df9]

Zhu 2018 *{published data only}*

NCT02202564. Preliminary results for the double-dose adenovirus-mediated adjuvant therapy improving outcome of liver transplantation in patients with advanced hepatocellular carcinoma. clinicaltrials.gov/ct2/show/NCT02202564 (first received 29 July 2014).

* Zhu R, Weng D, Lu S, Lin D, Wang M, Chen D, et al. Double-dose adenovirus-mediated adjuvant gene therapy improves liver transplantation outcomes in patients with advanced hepatocellular carcinoma. *Human Gene Therapy* 2018;**29**:251-8.

References to studies excluded from this review

Breitbach 2015 *{published data only}*

Breitbach CJ, Moon A, Burke J, Hwang Tae-Ho, Kirn DH. Chapter 19: a phase 2, open-label, randomized study of Pexa-Vec (JX-594) administered by intratumoral injection in patients with unresectable primary hepatocellular. In: Wolfgang Walther and Ulrike Stein, editors(s). Gene Therapy of Solid Cancers: Methods and Protocols, Methods in Molecular Biology. Vol. **1317**. New York: Springer Science+Business Media, 2015:343-57. [DOI: 10.1007/978-1-4939-2727-2_19]

Dong 2014 *{published data only}*

Dong J, Li W, Dong A, Mao S, Shen L, Li S, et al. Gene therapy for unresectable hepatocellular carcinoma using recombinant human adenovirus type 5. *Medical Oncology* 2014;**31**(8):95. [DOI: 10.1007/s12032-014-0095-4]

Guan 2011 *{published data only}*

Guan YS, Liu Y, He Q, Li X, Yang L, Hu Y, et al. p53 Gene therapy in combination with transcatheter arterial chemoembolization for HCC: one-year follow-up. *World Journal of Gastroenterology* 2011;**17**(16):2143-9. [DOI: 10.3748/wjg.v17.i16.2143]

Heo 2013 *{published data only}*

Heo J, Reid T, Ruo L, Breitbach CJ, Rose S, Bloomston M, et al. Randomized dose-finding clinical trial of oncolytic immunotherapeutic vaccinia JX-594 in liver cancer. *Nature Medicine* 2013;**19**(3):329-36.

Hernandez-Alcoceba 2006 *{published data only}*

Hernandez-Alcoceba R, Sangro B, Prieto J. Gene therapy of liver cancer. *World Journal of Gastroenterology* 2006;**12**(38):6085-97. [DOI: 10.3748/wjg.v12.i38.6085]

Jebar 2015 *{published data only}*

Jebar AH, Errington-Mais F, Vile RG, Selby PJ, Melcher AA, Griffin S. Progress in clinical oncolytic virus-based therapy for hepatocellular carcinoma. *Journal of General Virology* 2015;**986**:1533-50. [DOI: 10.1099/vir.0.000098]

Lencioni 2015 *{published data only}*

Lencioni R, Kim CW, Rose SC, Breitbach CJ, Burke JM, Hickman T, et al. Intratumoral injection of the oncolytic immunotherapeutic Pexa-Vec (JX-594) in liver tumors and hepatocellular carcinoma: recommendations for clinical practice. *European Journal of Cancer* 2015;**3**(51):S405-6.

Liu 2015 *{published data only}*

Liu Y, Zhang Y, Bautista D, Tang S, Zhou J, Li C, et al. Trans-arterial p53-gene-embolization with gelatin sponge microparticles for hepatocellular carcinoma with BCLC stage B: single-center experience. *Cell Biochemistry and Biophysics* 2015;**71**(1):99-104. [DOI: 10.1007/s12013-014-0167-2]

NCT02309788 *{published data only}*

NCT02309788. Radiotherapy in hepatocellular carcinomas after hepatectomy with narrow margin (<1 cm) or portal vein thrombosis (RHCC). clinicaltrials.gov/ct2/show/NCT02309788 (first received 5 December 2014).

Park 2008 *{published data only}*

Park BH, Hwang T, Liu TC, Sze DY, Kim JS, Kwon HC, et al. Use of a targeted oncolytic poxvirus, JX-594, in patients with refractory primary or metastatic liver cancer: a phase I trial.

Lancet Oncology 2008;**9**(6):533-42. [DOI: 10.1016/S1470-2045(08)70107-4]

Penuelas 2005 *{published data only}*

Penuelas I, Mazzolini G, Boán JF, Sangro B, Martí-Climent J, Ruiz M, et al. Positron emission tomography imaging of adenoviral-mediated transgene expression in liver cancer patients. *Gastroenterology* 2005;**128**(7):1787-95.

Qu 2020 *{published data only}*

Qu J, Lu W, Chen M, Gao W, Zhang C, Guo B, et al. Combined effect of recombinant human adenovirus p53 and curcumin in the treatment of liver cancer. *Experimental and Therapeutic Medicine* 2020;**20**(5):18. [DOI: 10.3892/etm.2020.9145]

Sangro 2010 *{published data only}*

Sangro B, Mazzolini G, Ruiz M, Ruiz J, Quiroga J, Herrero I, et al. A phase I clinical trial of thymidine kinase-based gene therapy in advanced hepatocellular carcinoma. *Cancer Gene Therapy* 2010;**17**(12):873-43.

Sangro 2014 *{published data only (unpublished sought but not used)}*

Sangro B, Palmer D, Melero I. Immunotherapy of hepatocellular carcinoma. *Hepatic Oncology* 2014;**1**(4):433-46. [DOI: 10.2217/hep.14.16]

Sangro 2021 *{published data only (unpublished sought but not used)}*

Sangro B, Sarobe P, Hervás-Stubbs S, Melero I. Advances in immunotherapy for hepatocellular carcinoma. *Nature Reviews Gastroenterology and Hepatology* 2021;**18**(8):525-43. [DOI: 10.1038/s41575-021-00438-0]

Schmitz 2002 *{published data only}*

Schmitz V, Qian C, Ruiz J, Sangro B, Melero I, Mazzolini G, et al. Gene therapy for liver diseases: recent strategies for treatment of viral hepatitis and liver malignancies. *Gut* 2002;**50**(1):130-5. [DOI: 10.1136/gut.50.1.130]

References to ongoing studies

NCT00003147 *{published data only}*

NCT00003147. Gene therapy in treating patients with cancer of the liver. clinicaltrials.gov/ct2/show/NCT00003147 (first received 24 August 2004).

NCT00300521 *{published data only}*

NCT00300521. Liver transplantation with ADV-TK gene therapy improves survival in patients with advanced hepatocellular carcinoma. www.clinicaltrials.gov/ct2/show/NCT00300521 (first received 9 March 2006).

NCT00451022 *{published data only}*

NCT00451022. Follow-up study of subjects previously enrolled in poxviral vector gene transfer studies. clinicaltrials.gov/ct2/show/NCT00451022 (first received 22 March 2007).

NCT01071941 *{published data only}*

NCT01071941. rRp450 – phase I trial in liver metastases and primary liver tumors. clinicaltrials.gov/ct2/show/NCT01071941 (first received 19 February 2010).

NCT01869088 *{published data only}*

NCT01869088. Phase III trial of transcatheter arterial chemoembolization (TACE) plus recombinant human adenovirus type 5 Injection for unresectable hepatocellular carcinoma (HCC). clinicaltrials.gov/ct2/show/NCT01869088 (first received 5 June 2013).

NCT02395250 *{published data only}*

NCT02395250. Anti-GPC3 CAR T for treating patients with advanced HCC. clinicaltrials.gov/ct2/show/NCT02395250 (first received 23 March 2015).

NCT02418988 *{published data only}*

NCT02418988. Trans-catheter chemo-embolization combined with rAd-p53 gene injection in treatment of advanced hepatocellular carcinoma. clinicaltrials.gov/ct2/show/NCT02418988 (first received 17 April 2015).

NCT02509169 *{published data only}*

NCT02509169. Trans-catheter arterial embolization combined with p53 gene therapy for treatment of advanced hepatocellular carcinoma. clinicaltrials.gov/ct2/show/NCT02509169 (first received 27 July 2015).

NCT02561546 *{published data only}*

NCT02561546. p53 gene therapy in treatment of diabetes concurrent with hepatocellular carcinoma. clinicaltrials.gov/ct2/show/NCT02561546 (first received 28 September 2015).

NCT02905188 *{published data only}*

NCT02905188. Glypican 3-specific chimeric antigen receptor expressing T cells for hepatocellular carcinoma (GLYCAR) (GLYCAR). clinicaltrials.gov/ct2/show/NCT02905188 (first received 19 September 2016).

NCT03313596 *{published data only}*

NCT03313596. Multicenter RCT of ADV-TK gene therapy improving the outcome of liver transplantation for advanced HCC. clinicaltrials.gov/ct2/show/NCT03313596 (first received 18 October 2017).

NCT03680560 *{published data only}*

NCT03680560. Study of ACTR T cell product in combination with trastuzumab in subjects with HER2-positive advanced solid tumor cancers. clinicaltrials.gov/ct2/show/NCT03680560 (first received 21 September 2018).

NCT04715191 *{published data only}*

NCT04715191. Interleukin-15 and -21 armored glypican-3-specific chimeric antigen receptor expressed in T cells for pediatric solid tumors. clinicaltrials.gov/ct2/show/NCT04715191 (first received 20 January 2021).

Additional references

Asrani 2019

Asrani SK, Devarbhavi H, Eaton J, Kamath PS. Burden of liver diseases in the world. *Journal of Hepatology* 2019;**70**(1):151-71.

Bray 2018

Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of Incidence and mortality worldwide for 36 cancers in 185 countries. *CA: A Cancer Journal for Clinicians* 2018;**68**:394-424. [DOI: 10.3322/caac.21492]

Bruix 2011

Bruix J, Sherman M. Management of hepatocellular carcinoma: an update. *Hepatology* 2011;**53**:1020-22.

Bruix 2016

Bruix J, Reig M, Sherman M. Evidence-based diagnosis, staging, and treatment of patients with hepatocellular carcinoma. *Gastroenterology* 2016;**150**(4):835-53.

Chakraborty 2022

Chakraborty E, Sarkar D. Emerging therapies for hepatocellular carcinoma (HCC). *Cancers (Basel)* 2022;**14**(11):2798. [DOI: 10.3390/cancers14112798]

Deeks 2022

Deeks JJ, Higgins JP, Altman DG. Chapter 10: Analysing data and undertaking meta-analyses. In: Higgins JP, Thomas J, Chandler J, Cumpston M, Li T, Page MJ, Welch VA, editor(s). Cochrane Handbook for Systematic Reviews of Interventions Version 6.3 (updated February 2022). Available from training.cochrane.org/handbook/archive/v6.3.

Delhove 2020

Delhove J, Osenk I, Prichard I, Donnelley M. Public acceptability of gene therapy and gene editing for human use: a systematic review. *Human Gene Therapy* 2020;**31**:20-46. [DOI: 10.1089/hum.2019.197]

Ding 2021

Ding J, Wen Z. Survival improvement and prognosis for hepatocellular carcinoma: analysis of the SEER database. *BMC Cancer* 2021;**21**(1):1157. [DOI: 10.1186/s12885-021-08904-3]

Dunbar 2018

Dunbar CE, High KA, Joung JK, Kohn DB, Ozawa K, Sadelain M. Gene therapy comes of age. *Science* 2018;**359**(6372):eaan4672. [DOI: 10.1126/science.aan4672]

El-Serag 2011

El-Serag HB. Hepatocellular carcinoma. *New England Journal of Medicine* 2011;**365**(12):1118-27.

ESMO 2021

European Society for Medical Oncology (ESMO) Guidelines Committee 2021. Updated treatment recommendations for hepatocellular carcinoma (HCC) from the ESMO clinical practice guidelines. www.esmo.org/guidelines/guidelines-by-topic/gastrointestinal-cancers/hepatocellular-carcinoma/eupdate-hepatocellular-carcinoma-treatment-recommendations (accessed 18 January 2023).

European Parliament 2007

European Parliament and Council. Regulation (EC) No 1394/2007 of the European Parliament and of the Council of 13 November 2007 on advanced therapy medicinal products and amending directive 2001/83/EC. *Official Journal of the European Union* 2007;**324**:121-37.

Fitzmaurice 2019

Fitzmaurice C, Abate D, Abbasi N, Abbastabar H, Abd-Allah F, Abdel-Rahman O, et al. Global, regional, and national cancer incidence, mortality, years of life lost, years lived with disability, and disability-adjusted life-years for 29 cancer groups, 1990 to 2017: a systematic analysis for the global burden of disease study. *JAMA Oncology* 2019;**5**(12):1749-68.

Forner 2018

Forner A, Reig M, Bruix J. Hepatocellular carcinoma. *Lancet* 2018;**391**:1301-14.

Fuks 2012

Fuks D, Dokmak S, Paradis V, Diouf M, Durand F, Belghiti J. Benefit of initial resection of hepatocellular carcinoma followed by transplantation in case of recurrence: an intention-to-treat analysis. *Hepatology* 2012;**55**(1):132-40.

Ginn 2018

Ginn SL, Amaya AK, Alexander IE, Edelstein M, Abedi MR. Gene therapy clinical trials worldwide to 2017: an update. *Journal of Gene Medicine* 2018;**20**(5):e3015.

GRADEpro GDT [Computer program]

GRADEpro GDT. Version accessed 9 January 2023. Hamilton (ON): McMaster University (developed by Evidence Prime), 2023. Available at gradepro.org.

Guo 2014

Guo XE, Ngo B, Modrek AS, Lee WH. Targeting tumor suppressor networks for cancer therapeutics. *Current Drug Targets* 2014;**15**(1):2-16. [DOI: 10.2174/1389450114666140106095151]

Hacein-Bey-Abina 2003

Hacein-Bey-Abina S, von Kalle C, Schmidt M, McCormack MP, Wulffraat N, Leboulch PA, et al. LMO2-associated clonal T cell proliferation in two patients after gene therapy for SCID-X1. *Science* 2003;**302**:415-9.

Hashimoto 2009

Hashimoto E, Yatsuji S, Tobari M, Taniai M, Torii N, Tokushige K, et al. Hepatocellular carcinoma in patients with nonalcoholic steatohepatitis. *Journal of Gastroenterology* 2009;**44**(19):89-95.

Hermiston 2002

Hermiston T, Kuhn I. Armed therapeutic viruses: strategies and challenges to arming oncolytic viruses with therapeutic genes. *Cancer Gene Therapy* 2002;**9**:1022-35. [DOI: 10.1038/sj.cgt.7700542]

Hernandez-Alcoceba 2006

Hernandez-Alcoceba R, Sangro B, Prieto J. Gene therapy of liver cancer. *World Journal of Gastroenterology* 2006;**12**(38):6085-97. [DOI: 10.3748/wjg.v12.i38.6085]

Higgins 2022a

Higgins JP, Thomas J, Chandler J, Cumpston M, Li T, Page M, Welch VA, editor(s). Cochrane Handbook for Systematic Reviews of Interventions Version 6.3 (updated February 2022). Cochrane, 2022. Available from training.cochrane.org/handbook/archive/v6.3.

Higgins 2022b

Higgins JP, Li T, Deeks JJ. Chapter 6: Choosing effect measures and computing estimates of effect. In: Higgins JP, Thomas J, Chandler J, Cumpston M, Li T, Page MJ, Welch VA, editor(s). Cochrane Handbook for Systematic Reviews of Interventions Version 6.3 (updated February 2022). Available from training.cochrane.org/handbook/archive/v6.3.

Higgins 2022c

Higgins JP, Savović J, Page MJ, Elbers RG, Sterne JA. Chapter 8: Assessing risk of bias in a randomized trial. In: Higgins JP, Thomas J, Chandler J, Cumpston M, Li T, Page MJ, Welch VA, editor(s). Cochrane Handbook for Systematic Reviews of Interventions Version 6.3 (updated February 2022). Available from training.cochrane.org/handbook/archive/v6.3.

Higgins 2023

Higgins JP, Eldridge S, Li T. Chapter 23: Including variants on randomized trials. In: Higgins JP, Thomas J, Chandler J, Cumpston M, Li T, Page MJ, Welch VA, editor(s). Cochrane Handbook for Systematic Reviews of Interventions Version 6.4 (updated August 2023). Available from training.cochrane.org/handbook/current.

Hollis 1999

Hollis S, Campbell F. What is meant by intention to treat analysis? Survey of published randomised controlled trials. *BMJ (Clinical Research Ed.)* 1999;**319**(7211):670-4.

Hughes 2012

Hughes J, Alusi G, Wang Y. Gene therapy and nasopharyngeal carcinoma. *Rhinology* 2012;**50**(2):115-21.

ICH-GCP 2016

International Council for Harmonisation of technical requirements for pharmaceuticals for human use (ICH). ICH Harmonised Guideline. Integrated addendum to ICH E6(R1): guideline for good clinical practice E6(R2). database.ich.org/sites/default/files/E6_R2_Addendum.pdf (accessed 18 July 2022).

Jakobsen 2014

Jakobsen JC, Gluud C, Winkel P, Lange T, Wetterslev J. The thresholds for statistical and clinical significance – a five-step procedure for evaluation of intervention effects in randomised clinical trials. *BMC Medical Research Methodology* 2014;**14**:34. [DOI: 10.1186/1471-2288-14-34]

Keus 2010

Keus F, Wetterslev J, Gluud C, van Laarhoven CJ. Evidence at a glance: error matrix approach for overviewing available evidence. *BMC Medical Research Methodology* 2010;**10**:90. [DOI: 10.1186/1471-2288-10-90]

Kumar 2014

Kumar M, Panda D. Role of supportive care for terminal stage hepatocellular carcinoma. *Journal of Clinical and Experimental Hepatology* 2014;**Suppl 3**:S130-9. [DOI: 10.1016/j.jceh.2014.03.049]

Lee 2017

Lee CS, Bishop ES, Zhang R, Yu X, Farina EM, Yan S, et al. Adenovirus-mediated gene delivery: potential applications for gene and cell-based therapies in the new era of personalized medicine. *Genes and Diseases* 2017;**4**(2):43-63. [DOI: 10.1016/j.gendis.2017.04.001]

Lefebvre 2011

Lefebvre C, Manheimer E, Glanville J, on behalf of the Cochrane Information Retrieval Methods Group. Chapter 6: Searching for studies. In: Higgins JP, Green S, editor(s). Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0 (updated March 2011). The Cochrane Collaboration, 2011. Available from training.cochrane.org/handbook/archive/v5.1/.

Lefebvre 2022

Lefebvre C, Glanville J, Briscoe S, Littlewood A, Marshall C, Metzendorf M-I, et al. Chapter 4: Searching for and selecting studies. In: Higgins JP, Thomas J, Chandler J, Cumpston M, Li T, Page MJ, Welch VA, editor(s). Cochrane Handbook for Systematic Reviews of Interventions Version 6.3 (updated February 2022). Available from training.cochrane.org/handbook/archive/v6.3.

Li 2005a

Li H, Fu X, Chen Y, Hong Y, Tan Y, Cao H, et al. Use of adenovirus-delivered siRNA to target oncoprotein p28GANK in hepatocellular carcinoma. *Gastroenterology* 2005;**128**(7):2029-41. [DOI: 10.1053/j.gastro.2005.03.001]

Li 2005b

Li L, Liu RY, Huang JL, Liu QC, Li Y, Wu PH, et al. Adenovirus-mediated intra-tumoral delivery of the human endostatin gene inhibits tumor growth in nasopharyngeal carcinoma. *International Journal of Cancer* 2005;**118**(8):2064-71. [DOI: 10.1002/ijc.21585]

Lisa 2022

Lisa B. Stamp out fake clinical data by working together. *Nature* 2022;**601**:167.

Liu 2022

Liu B, Zhang Y, Chen H, Li W, Tsochatzis E. The combination of transcatheter arterial chemoembolisation (TACE) and thermal ablation versus TACE alone for hepatocellular carcinoma. *Cochrane Database of Systematic Reviews* 2022, Issue 1. Art. No: CD013345. [DOI: 10.1002/14651858.CD013345.pub2]

Llovet 2021

Llovet JM, Kelley RK, Villanueva A, Singal AG, Pikarsky E, Roayaie S, et al. Hepatocellular carcinoma. *Nature Reviews Disease Primers* 2021;**7**(1):6. [DOI: 10.1038/s41572-020-00240-3]

Lundh 2017

Lundh A, Lexchin J, Mintzes B, Schroll JB, Bero L. Industry sponsorship and research outcome. *Cochrane Database of Systematic Reviews* 2017, Issue 2. Art. No: MR000033. [DOI: 10.1002/14651858.MR000033.pub3]

Maeder 2016

Maeder ML, Gersbach CA. Genome editing technologies for gene and cell therapy. *Molecular Therapy* 2016;**24**(3):430-46. [DOI: 10.1038/mt.2016.10.]

Marrero 2018

Marrero JA, Kulik LM, Sirlin CB, Zhu AX, Finn RS, Abecassis MM, et al. Diagnosis, staging, and management of hepatocellular carcinoma: 2018 practice guidance by the American Association for the Study of Liver Diseases. *Hepatology* 2018;**68**(2):723-50. [DOI: 10.1002/hep.29913]

McKenzie 2022

McKenzie JE, Brennan SE. Chapter 12: Synthesizing and presenting findings using other methods. In: Higgins JP, Thomas J, Chandler J, Cumpston M, Li T, Page MJ, Welch VA, editor(s). Cochrane Handbook for Systematic Reviews of Interventions Version 6.3 (updated February 2022). Available from training.cochrane.org/handbook/archive/v6.3.

Micklus 2018

Micklus A. Gene therapy: a paradigm shift in medicine. pharmaintelligence.informa.com/~/media/informa-shop-window/pharma/whitepapers/dmhc-gene-therapy-whitepaper.pdf (accessed 18 January 2023).

Miller 2005

Miller DG, Trobridge GD, Petek LM, Jacobs MA, Kaul R, Russell DW. Large-scale analysis of adeno-associated virus vector integration sites in normal human cells. *Journal of Virology* 2005;**79**(17):11434-42.

Namba 2012

Namba M, Tsunemi Y, Kawashima Ml. Sorafenib-induced erythema multiforme: three cases. *European Journal of Dermatology* 2012;**21**(6):1015-6.

Naso 2017

Naso MF, Tomkowicz B, Perry WL 3rd, Strohl WR. Adeno-associated virus (AAV) as a vector for gene therapy. *BioDrugs* 2017;**31**(4):317-34. [DOI: 10.1007/s40259-017-0234-5]

Nathwani 2014

Nathwani AC, Reiss UM, Tuddenham EG, Rosales C, Chowdary P, McIntosh J, et al. Long-term safety and efficacy of factor IX gene therapy in hemophilia B. *New England Journal of Medicine* 2014;**371**(21):1994-2004. [DOI: 10.1056/NEJMoa1407309] [PMID: 25409372]

Newel 1992

Newell DJ. Intention-to-treat analysis: implications for quantitative and qualitative research. *International Journal of Epidemiology* 1992;**21**(5):837-41.

NICE 2021

National Institute for Health and Care Excellence. NICE final draft guidance approves life-changing gene therapy for treating spinal muscular atrophy. www.nice.org.uk/news/article/nice-approves-life-changing-gene-therapy-for-treating-spinal-muscular-atrophy (accessed 18 January 2023).

Oliveri 2011

Oliveri RS, Wetterslev J, Gluud C. Transarterial (chemo)embolisation for unresectable hepatocellular carcinoma. *Cochrane Database of Systematic Reviews* 2011, Issue 3. Art. No: CD004787. [DOI: 10.1002/14651858.CD004787.pub2]

Olowoyeye 2020

Olowoyeye A, Okwundu CI. Gene therapy for sickle cell disease. *Cochrane Database of Systematic Reviews* 2020, Issue 11. Art. No: CD007652. [DOI: 10.1002/14651858.CD007652.pub7]

Ottaviano 2017

Ottaviano M, Palmieri G, Damiano V, Tortora M, Montella L. Rescue of sorafenib-pretreated advanced hepatocellular carcinoma with tamoxifen. *Clinical Research and Trial* 2017;**3**(6):1-6. [DOI: 10.15761/CRT.1000200]

Page 2021a

Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ (Clinical Research Ed.)* 2021;**372**:n71.

Page 2021b

Page MJ, Moher D, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. PRISMA 2020 explanation and elaboration: updated guidance and exemplars for reporting systematic reviews. *BMJ (Clinical Research Ed.)* 2021;**372**:n160.

Parkin 2001

Parkin DM, Bray F, Ferlay J, Pisani P. Estimating the world cancer burden: Globocan 2000. *International Journal of Cancer* 2001;**94**(2):153-6.

Parmar 1998

Parmar MK, Torri V, Stewart L. Extracting summary statistics to perform meta-analyses of the published literature for survival endpoints. *Statistics in Medicine* 1998;**17**(24):2815-34.

Perz 2006

Perz JF, Armstrong GL, Farrington LA, Hutin YJ, Bell BP. The contributions of hepatitis B virus and hepatitis C virus infections to cirrhosis and primary liver cancer worldwide. *Journal of Hepatology* 2006;**45**:529-38.

Petrick 2016a

Petrick JL, Braunlin M, Laversanne M, Valery PC, Bray F, McGlynn KA. International trends in liver cancer incidence,

overall and by histologic subtype, 1978–2007. *International Journal of Cancer* 2016;**139**:1534-45.

Petrick 2016b

Petrick JL, Kelly SP, Altekruse SF, McGlynn KA, Rosenberg PS. Future of hepatocellular carcinoma incidence in the United States forecast through 2030. *Journal of Clinical Oncology* 2016;**34**:1787-94.

Petrick 2018

Petrick JL, Campbell PT, Koshiol J, Thistle JE, Andreotti G, Beane-Freeman LE, et al. Tobacco, alcohol use and risk of hepatocellular carcinoma and intrahepatic cholangiocarcinoma: the liver cancer pooling project. *British Journal of Cancer* 2018;**118**(7):1005-12.

Qian 2000

Qian C, Drozdzik M, Caselmann WH, Prieto J. The potential of gene therapy in the treatment of hepatocellular carcinoma. *Journal of Hepatology* 2000;**32**(2):344-51.

Reghupaty 2019

Reghupaty SC, Sarkar D. Current status of gene therapy in hepatocellular carcinoma. *Cancers* 2019;**11**(9):1265. [DOI: 10.3390/cancers11091265]

RevMan 2020 [Computer program]

Review Manager (RevMan). Version 1.22.0. The Cochrane Collaboration, 2020. Available at revman.cochrane.org.

Sandig 1997

Sandig V, Brand K, Herwig S, Lukas J, Bartek J, Strauss M. Adenovirally transferred p16INK4/CDKN2 and p53 genes cooperate to induce apoptotic tumor cell death. *Nature Medicine* 1997;**3**:313-9.

Savovic 2018

Savovic J, Turner RM, Mawdsley D, Jones HE, Beynon R, Higgins JP, et al. Association between risk-of-bias assessments and results of randomized trials in Cochrane reviews: the ROBES meta-epidemiologic study. *American Journal of Epidemiology* 2018;**187**(5):1113-22. [DOI: 10.1093/aje/kwx344]

Schünemann 2022

Schünemann HJ, Higgins JP, Vist GE, Glasziou P, Akl EA, Skoetz N, et al. Chapter 14: Completing 'Summary of findings' tables and grading the certainty of the evidence. In: Higgins JP, Thomas J, Chandler J, Cumpston M, Li T, Page MJ, Welch VA, editor(s). Cochrane Handbook for Systematic Reviews of Interventions Version 6.3 (updated February 2022). Available from training.cochrane.org/handbook/archive/v6.3.

Shi 2014

Shi L, Feng Y, Lin H, Ma R, Cai X. Role of estrogen in hepatocellular carcinoma: is inflammation the key? *Journal of Translational Medicine* 2014;**12**:93. [DOI: 10.1186/1479-5876-12-93]

Singal 2023

Singal AG, Llovet JM, Yarchoan M, Mehta N, Heimbach JK, Dawson LA, et al. AASLD Practice Guidance on prevention, diagnosis, and treatment of hepatocellular carcinoma. Erratum in PMID: 37199193. *Hepatology* 2023;**78**(6):E105. [DOI: 10.1097/HEP.0000000000000466]

Smith 2015

Smith RP, Riordan JD, Feddersen CR, Dupuy AJ. A hybrid adenoviral vector system achieves efficient long-term gene expression in the liver via piggyBac transposition. *Human Gene Therapy* 2015;**26**(6):377-85. [DOI: 10.1089/hum.2014.123]

Storebø 2018

Storebø OJ, Pedersen N, Ramstad E, Kielsholm ML, Nielsen SS, Krogh HB, et al. Methylphenidate for attention deficit hyperactivity disorder (ADHD) in children and adolescents – assessment of adverse events in non-randomised studies. *Cochrane Database of Systematic Reviews* 2018, Issue 5. Art. No: CD012069. [DOI: 10.1002/14651858.CD012069.pub2]

Thorlund 2017

Thorlund K, Engstrøm J, Wetterslev J, Brok J, Imberger G, Gluud C. User Manual for Trial Sequential Analysis (TSA); 2nd edition. Copenhagen Trial Unit, 2017. Available from ctu.dk/tsa/learn-more (accessed 18 July 2022).

Tierney 2007

Tierney JF, Stewart LA, Ghersi D, Burdett S, Sydes MR. Practical methods for incorporating summary time-to-event data into meta-analysis. *Trials* 2007;**8**(1):16. [DOI: 10.1186/1745-6215-8-16]

TSA 2021 [Computer program]

TSA – Trial Sequential Analysis. Version 0.9.5.10 Beta. Copenhagen: Copenhagen Trial Unit, Centre for Clinical Intervention Research, The Capital Region, Copenhagen University Hospital – Rigshospitalet, 2021. ctu.dk/tsa/downloads/.

Uccello 2012

Uccello M, Malaguarnera G, Corriere T, Biondi A, Basile F, Malaguarnera M. Risk of hepatocellular carcinoma in workers exposed to chemicals. *Hepatitis Monthly* 2012;**12**(10):e5943. [DOI: 10.5812/hepatmon.5943]

US National Health Library 2022

US National Health Library. How does gene therapy work? medlineplus.gov/genetics/understanding/therapy/procedures/ (accessed 18 January 2023).

Vitale 2023

Vitale A, Cabibbo G, Iavarone M, Viganò L, Pinato DJ, Ponziani FR, et al, HCC Special Interest Group of the Italian Association for the Study of the Liver. Personalised management of patients with hepatocellular carcinoma: a multiparametric therapeutic hierarchy concept. *Lancet Oncology* 2023;**24**(7):e312-22. [DOI: 10.1016/S1470-2045(23)00186-9]

Wetterslev 2017

Wetterslev J, Jakobsen JC, Gluud C. Trial Sequential Analysis in systematic reviews with meta-analysis. *BMC Medical Research Methodology* 2017;**17**(1):39. [DOI: 10.1186/s12874-017-0315-7]

Wetterslev 2009

Wetterslev J, Thorlund K, Brok J, Gluud C. Estimating required information size by quantifying diversity in a random-effects meta-analysis. *BMC Medical Research Methodology* 2009;**9**:86.

Ye 2016

Ye SL, Chen X, Yang J, Bie P, Zhang S, Liu F, et al. Safety and efficacy of sorafenib therapy in patients with hepatocellular carcinoma: final outcome from the Chinese patient subset of the GIDEON study. *Oncotarget* 2016;**7**:6. [DOI: 10.18632/oncotarget.6781]

CHARACTERISTICS OF STUDIES

Characteristics of included studies *[ordered by study ID]*

Chen 2014

Study characteristics	
Methods	Randomised controlled trial, prospective **Site:** Cancer Hospital, District XuanWu, Nan Jing City, Jiangsu Province, China **Study period:** May 2005 to January 2009 **Clinical trial registry:** no information
Participants	48 participants with advanced HCC **Age range** Intervention group: 35–76 years (n = 30) Control group: 28–68 years (n = 18) **Male, n (%)** Intervention group: 22/30 (73.3%) Control group: 14/18 (77.8%) **Inclusion criteria** • Men and women aged 18–75 years • Confirmed diagnosis of primary HCC according to the Clinical Diagnosis and Staging Criteria of Primary HCC developed by the Chinese Society of Liver Cancer • Diffuse type or > 10 cm tumour diameter • Hepatic function classified as Child-Pugh B or more severe • ALT > 4-fold increase • BCLC stage of HCC of B or more • ECOG PS 0–2 • WBC > 4.0 × 10^9/L, haemoglobin > 100 g/L, platelet count > 80 × 10^9/L • Increased AFP (> 400 ng/mL) • Signed the informed consent **Exclusion criteria** • Not mentioned
Interventions	**2 groups**

References to other published versions of this review

Naing 2020

Naing C, Leong C-O, Aung HH, Mai C-W, Chan EW, Kew ST. Gene therapy for people with hepatocellular carcinoma. *Cochrane Database of Systematic Reviews* 2020, Issue 9. Art. No: CD013731. [DOI: 10.1002/14651858.CD013731]

* Indicates the major publication for the study

Chen 2014 *(Continued)*

	Intervention: Ad-p53 + hydroxycamptothecin 20 mg once a week for 3 weeks • Ad-p53 injection: Gendicine (Shenzhen SiBiono GenTech Co, Ltd) **Control:** hydroxycamptothecin 20 mg, once a week for 3 weeks
Outcomes	**Relevant to this review** • Survival • Disease progression • Change in liver function test **Not relevant to this review** • Karnofsky PS (scores of participants who have or have not completed a course of treatment) • Change of lesion (measured with enhanced CT) • AFP values
Notes	**Funding:** not mentioned **Conflict of interests:** (quote) "All patients in this study signed the same informed consent." Email sent to the author on 18 April 2021 Did not receive a reply **Sources obtained for risk of bias assessment:** journal article with results of the trial

Habib 2002

Study characteristics	
Methods	Randomised controlled, open label, prospective **Site:** Kasr El -Eini Hospital in Cairo, Egypt **Study period:** November 1998 to August 1999 **Clinical trial registry:** no information
Participants	10 participants with histologically confirmed HCC (2 with posthepatitis liver cirrhosis classified as Child B, and 8 with Child A). **Age range** Intervention group: 50–65 years (n = 5) Control group: 50–74 years (n = 5) **Male, n (%)** Intervention group: 5 (100) Control group: 3 (60) **Inclusion criteria** • Men and women aged 35–75 years • Histologically diagnosis of HCC • No more than 2 tumours

Habib 2002 *(Continued)*

- Life expectancy ≥ 3 months
- Adequate performance status (Karnofsky score > 70%)
- WBC > 3000/µL, platelet count > 50,000 µ/L, haematocrit > 25%, prothrombin time < 20 seconds, creatinine 1.8 mg/dL, total bilirubin < 5 mg/dL, AST and ALT < 10 times upper limit of normal (normal levels 40 IU/L)
- Signed the informed consent

Exclusion criteria

- Pregnant women
- Fertile patients unless using effective contraception for ≥ 1 month before study entry
- Uncontrolled serious bacterial, viral, fungal, or parasitic infection systemic corticosteroid therapy or other immunosuppressive therapy administered within the last 3 months

Interventions	**2 groups** **Intervention:** gene therapy with E1B-deleted (dl1520) adenovirus (n = 5) Sterile 95% ethanol was administered with an 18- to 20-gauge spinal needle using the Livraghi technique. **Control:** percutaneous ethanol injection (n = 5)
Outcomes	**Relevant to this review** • Disease progression • Adverse events (number of participants) • Changes in liver function tests **Not relevant to this review** • Detection of adenovirus type 5 sequence using polymerase chain reaction test • Mean level of anti-adenovirus type 5 antibody
Notes	**Funding:** Pedersen Foundation **Conflict of interests:** (quote) "Before signing an informed consent document, a patient information leaflet was given to each patient, which explained the experimental nature of the study, risks involved and the unlikelihood of potential benefit to the individual." p.255 "approval by the Scientific and Ethical Committee of Cairo University Medical School." p.255 Email resent to the author on 27 July 2022 Did not receive a reply **Sources obtained for risk of bias assessment:** journal article with results of the trial.

Li 2007

Study characteristics	
Methods	Randomised clinical trial, parallel-group design **Site:** 2 study sites Beijing Transplantation Center, ChaoYang Hospital and No. 180 Hospital of People's Liberation Army, Quanzhou, China **Study period:** September 2000 to October 2006

Li 2007 *(Continued)*

	Clinical trial registry: no information
Participants	45 with advanced and unresectable HCC **Mean age** Intervention group: 44.3 years (range 32–61) (n = 23) Control group: 43.9 years (range 26–65) (n = 22) **Male, n (%)** Intervention group: 23 (100) Control group: 20 (90.9) **Inclusion criteria** • Unresectable HCC > 5 cm, with no metastasis in lungs and bone **Exclusion criteria** • Distant metastasis detected by CT or MRI scan or at the time of surgery
Interventions	**2 groups** **Intervention:** liver transplantation plus ADV-TK therapy (5.0×10^{11} virus particles) during the operation (n = 23) **Control:** liver transplantation only (n = 22) Follow-up: median follow-up for the 45 participants was 26 months (range 2–50 months)
Outcomes	**Relevant to this review** • Overall survival rates • Adverse effects **Not relevant to this review** • Recurrence-free survival
Notes	**Funding:** National Science Foundation of China grants 30672227, 30600667, 30571950, 30271358, and 30370657 and "973" Program of China grant 2002CB513100 **Conflict of interests:** (quote) "inform consents were received from the patients." Protocol was approved by the Ethic committee with the provisions of the Declaration of Helsinki and local laws and regulations. Email sent to the author on 6 September 2021, and to the editor on 12 September 2021 Did not receive a reply **Sources obtained for risk of bias assessment:** journal article with results of the trial Regarding adverse events not considered serious, we could not obtain the Supplements mentioned in the trial publication, but the following is a citation from the publication: "ADV-TK treatment was well tolerated, and no significant toxicity was evident. Mild catarrhal symptoms were reported in 10 of 23 patients who received ADV-TK therapy. Slight fever with no chills was also observed after injection of ADV-TK in the first 3 days in the same patients. The temperatures ranged from 37.3°C to 38.3°C. The same 10 patients also suffered from light headache. All these symptoms subsided in 5 days. There was no evidence of liver or kidney dysfunction caused by ADV-TK in our study. Despite the abnormality of liver and renal function were observed in patients who received LT, especially in the first 2 weeks after operation, no added liver and renal toxicities were observed

Li 2007 *(Continued)*

	after injection of ADVTK (Supplementary Data 3; Supplementary Table S3). There were no significant differences of liver and renal function tests between LT-only group and LT plus ADV-TK group (Supplementary Data 3; Supplementary Table S3)."

Moehler 2019

Study characteristics	
Methods	Randomised, open-label Phase IIb trial (TRAVERSE) Intention-to-treat analysis **Site:** multiple; 25% of participants were from North America, 54% from Asia, 21% from Europe **Study period:** 24 October 2011 to 4 June 2013 **Clinical trial registry:** NCT01387555
Participants	129 participants with advanced HCC, whose tumour had progressed on or after sorafenib treatment or who were intolerant to sorafenib (TRAVERSE) **Mean age** Intervention group: 60 (SD 11) years (n = 86) Control group: 55 (SD 12) years (n = 43) **Male, n (%)** Intervention group: 72 (84) Control group: 33 (77) **Inclusion criteria** • Histological or clinical diagnosis of advanced HCC, who had radiographic progression on or after sorafenib treatment, or who were intolerant to sorafenib • Liver function of Child-Pugh Class A or B7 (without ascites), an ECOG PS ≤ 2, and adequate haematological, hepatic, and renal function • BCLC C-advanced: 75 (87%) in intervention group vs 34 (79%) in control group • Participants exhibited a high tumour burden in the liver, with a median sum of the longest diameters 104 mm (p.e1615817-2) **Exclusion criteria** (taken from a registered clinical trial as these criteria were not stated in the published article) • Received sorafenib within 14 days prior to randomisation • Received systemic anticancer therapy other than sorafenib within 28 days of randomisation • Prior treatment with JX-594 • Platelet count < 50,000/mm^3 • Total WBC < 2000 cells/mm^3 • Prior or planned organ transplant • Known significant immunodeficiency due to underlying illness (e.g. HIV/AIDS) or medication, or both • Severe or unstable cardiac disease • Viable central nervous system malignancy associated with clinical symptoms • Pregnant or nursing an infant

Moehler 2019 *(Continued)*

	• History of inflammatory skin condition (e.g. eczema requiring previous treatment, atopic dermatitis)
Interventions	**2 groups** (129 participants were randomly assigned 2:1) **Intervention:** Pexa-Vec plus BSC (n = 86) Pexa-Vec was given as a single intravenous infusion followed by up to 5 intratumoural injections. **Control:** BSC alone (n = 43)
Outcomes	**Primary outcomes** • Overall survival/all-cause mortality Secondary outcomes • Time to progression • Overall response rate • Disease control rate assessed by mRECIST (modified Response Evaluation Criteria In Solid Tumors) for HCC • Time to symptomatic progression • Safety • Tolerability • Quality of life
Notes	**Funding:** Jennerex Biotherapeutics Inc, San Francisco, USA (now Sillajen Biotherapeutics) and Transgene S.A., Illkirch-Graffenstaden, France **Conflict of interests:** disclosed **Sources obtained for risk of bias assessment:** journal article with results of the trial and non-commercial trial registry The trial included (quote) "patients whose tumor had progressed on/after sorafenib treatment or who were intolerant to sorafenib …" The trial authors wrote: "… this is the first large randomized trial of an oncolytic immunotherapy in HCC patients and the second world-wide late-stage, randomized trial of an oncolytic immunotherapy."

Tian 2009

Study characteristics	
Methods	Pilot phase 2 trial **Site:** Shenzhen Second People's Hospital of South Medical University in China **Study period:** October 2004 to February 2007 **Clinical registry:** no information. Corresponding author was contacted but did not respond.
Participants	46 participants with unresectable histologically confirmed HCC **Median age** Intervention group: 55 years (range 32–76) (n = 23) Control group: 56 years (range 33–71) (n = 23) **Male, n (%)**

Tian 2009 *(Continued)*

	Intervention group: 17 (74) Control group: 19 (83) ECOG PS 0: 16 (70%)/18 (78%); 1: 7 (30%)/5 (22%) Child–Pugh status: A: 16 (70%)/17 (74%); B: 7 (30%)/6 (26%) AFP (ng/mL): > 400: 17 (74%)/18 (80%); r400: 6 (26%)/5 (20%) Positive hepatitis status: hepatitis B: 20 (87%)/21 (91%); hepatitis C: 2 (9%)/1 (4%) H/O previous therapy: 11/8 **Inclusion criteria** • Aged 20–75 years • Histologically confirmed HCC, unresectable or refractory to standard therapies such as percutaneous ethanol injection, operation, TACE, radiofrequency ablation, or chemotherapy • ECOG PS 0 or 1 • No main portal vein involvement or extrahepatic metastasis • Life expectancy > 12 weeks • Adequate bone marrow, liver, and renal function **Exclusion criteria:** not mentioned
Interventions	**2 groups** **Intervention:** TACE+rAd-p53/5-Fu (n = 23) • Ad-p53 injection: Gendicine (Shenzhen SiBiono GenTech) **Control:** TACE alone (n = 23)
Outcomes	**Relevant to this review** • Disease progression • Safety and tolerability (according to the National Cancer Institute's Common Toxicity Criteria version 2 for a minimum of 12 months or until death) **Not relevant to this review** • Tumour response • Partial response • Stable response > 16 weeks
Notes	**Funding:** Science and Technology Grants for Medicine and Health Research from the Shenzhen Bureau of Science, Technology and Information, grant # 04029 **Conflict of interest:** no information Email sent to the author on 1 August 2021 and to the editor on 11 September 2021. Did not receive a reply **Sources obtained for risk of bias assessment:** journal article with results of the trial

Zhu 2018

Study characteristics

Zhu 2018 *(Continued)*

Methods	Randomised clinical trial **Site:** 2 sites, YouAn Hospital in Beijing and Chaoyang Hospital in Beijing in China **Study period:** October 2006 to December 2011 **Clinical registry:** NCT02202564
Participants	86 participants with advanced unresectable HCC **Mean age** Intervention group: 50.4 years (range 26–68) (n = 43) Control group: 49.6 years (27–68 range) (n = 43) **Male, n (%)** Intervention group: 37 (86) Control group: 34 (79) **Inclusion criteria** • Not received radiotherapy, chemotherapy, targeted therapy, or other biological treatment within 4 weeks before liver transplantation • Unresectable HCC with a single tumour > 5 cm in diameter or multiple tumours each > 3 cm in diameter • No metastases in the lungs and bones **Exclusion criteria** • Displayed 50% invasion of the main portal vein • People with cancer lesions beyond the upper half of the main portal vein
Interventions	**2 groups** **Intervention:** double-dose ADV-TK/GCV plus liver transplantation **Control:** liver transplantation only (i.e. orthotopic liver transplantation and subsequent immunosuppression therapy) (ADV-TK/GCV, manufactured by Tian Dakang Co) 1st ADV-TK dose was administered during liver transplantation surgery; 1.0×10^{12} viral particles of ADV-TK in 100 mL of 0.9% saline 2nd ADV-TK dose was administered 30 days after liver transplantation; 1.0×10^{12} viral particles of ADV-TK in 100 mL of 0.9% saline
Outcomes	**Relevant to this review** • Recurrence-free survival • Overall survival/all-cause mortality (Measured from the day of randomisation to death. For participants remaining alive, survival was recorded at the time of the last follow-up.) **Not relevant to this review** • Recurrence-free survival

Zhu 2018 *(Continued)*	
	(Measured from the day of randomisation to objective recurrence or HCC-related death).
Notes	**Funding:** Foundation for the Excellent Medical Staff of Beijing (2011-3-034), National Major Scientific and Technological Special Project for "Significant New Drugs Development" during the Twelfth Five-year Plan Period (2011ZX09101-001-10 and 2012ZX100002017-009), Beijing Municipal Administration of Hospitals Clinical Medicine Development of Special Funding Support (ZY201311), National Natural Science Fund of China (81090414, 81230038, 81472783, and 81025011), National Basic Research Program of China (973 Program; 2015CB553903); Chinese National Key Plan of Precision Medicine Research (2016YFC0902901), and National Science-Technology Supporting Plan Projects (2015BAI13B05). **Conflict of interest:** declared no conflicts of interest **Sources obtained for risk of bias assessment:** journal article with results of the trial and non-commercial trial registry

ADV-TK therapy: adenovirus-thymidine kinase; ADV-TK/GCV: adenovirus-thymidine kinase with ganciclovir therapy; AFP: alpha-fetoprotein; ALT: alanine aminotransferase; AST: aspartate aminotransferase; BCLC: Barcelona Clinic Liver Cancer; BSC: best supportive care; CT: computed tomography; ECOG: Eastern Cooperative Oncology Group; ECOG PS: Eastern Cooperative Oncology Group performance status; H/O: hydrogen/oxygen; HCC: hepatocellular carcinoma; IU: international units; MRI: magnetic resonance imaging; n: number of participants; Pexa-Vec: pexastimogene devacirepvec; rAd-p53/5-Fu: recombinant adenovirus-p53/5-fluorouracil; TACE: transarterial chemoembolisation; WBC: white blood cell.

Characteristics of excluded studies *[ordered by study ID]*

Study	Reason for exclusion
Breitbach 2015	No comparator of placebo or alternative intervention (within comparison of gene therapy intervention)
Dong 2014	Not a randomised trial, a retrospective study
Guan 2011	Not a randomised trial, a retrospective study, p.2144
Heo 2013	No comparator of placebo or alternative intervention (within comparison of gene therapy intervention)
Hernandez-Alcoceba 2006	Review article
Jebar 2015	Review article
Lencioni 2015	A summary report of 4 studies. Not an individual randomised trial
Liu 2015	Not a randomised trial (data on adverse events are extracted in a narrative format only)
NCT02309788	Not with gene therapy, only with radiotherapy
Park 2008	Participants were with other malignancies, and no separate data for HCC No comparator of placebo or alternative intervention (within comparison of gene therapy intervention)
Penuelas 2005	No comparator of placebo or alternative intervention (within comparison of gene therapy intervention)
Qu 2020	Not a randomised trial. Not with human participants. A laboratory-based study with cell lines.

Study	Reason for exclusion
Sangro 2010	Not a randomised trial. Single-arm study. Authors responded to our enquiries on 17 August 2021 (NCT00844623)
Sangro 2014	Review article
Sangro 2021	Review article
Schmitz 2002	Review article

HCC: hepatocellular carcinoma.

Characteristics of ongoing studies *[ordered by study ID]*

NCT00003147

Study name	Phase I study of percutaneous injections of adeno-virus p53 construct (ADENO-p53) for hepatocellular carcinoma
Methods	Phase I trial, single group assignment, open label
Participants	30 participants with liver cancer that cannot be surgically removed.
Interventions	Insertion of Adeno-Virus p53 Construct (ADENO-p53) gene into tumour
Outcomes	Not available
Starting date	February 1998
Contact information	Chandra P Belani at University of Pittsburgh, USA
Notes	Unique protocol ID: CDR0000065932 Status: terminated; contacted authors, but received no response Last update: 5 February 2013 Actual completion date: June 2003 Publication not found; contacted author, email address was inactive and received no response.

NCT00300521

Study name	Liver transplantation with ADV-TK gene therapy improves survival in patients with advanced hepatocellular carcinoma
Methods	Phase 2 trial, randomised, parallel assignment, open label Inclusion criteria • Clinical diagnosis of advanced HCC with no metastasis in lungs and bones • Accept liver transplantation Exclusion criteria • Small HCC

NCT00300521 *(Continued)*

	• Advanced hepatocellular with metastasis in lungs and bones
Participants	40 participants Age: child, adult, older adult
Interventions	ADV-TK (adenovirus-thymidine kinase enzyme)
Outcomes	Primary outcomes • Overall survival rate at 1 year • Overall survival rate at 2 years • Overall survival rate at 3 years • Overall recurrence-free survival rate at 1 year • Overall recurrence-free survival rate at 2 years • Overall recurrence-free survival rate at 3 years Secondary outcomes • AFP level before and after liver transplantation • Age on survival rate and recurrence-free survival rate • TNM stage on survival rate and recurrence-free survival rate • Child-Pugh classification on survival rate and recurrence-free survival rate • Vascular invasion on survival rate and recurrence-free survival rate
Starting date	9 March 2006
Contact information	Ding Ma at Cancer Biology Research Center, Tongji Hospital, Tongji Medical college, Huazhong University of Science and Technology, Wuhan, China
Notes	Status: completed Last update: 9 March 2006 Completion date: November 2005 Publication not found; author's e-mail address not available

NCT00451022

Study name	Follow-up of study subjects previously enrolled in immunotherapy studies utilizing gene transfer or other immunotherapeutic agent
Methods	Prospective observation of cases, non-probability sample Inclusion criteria • Subjects who received poxviral vectors (vaccinia or fowlpox, or both) or other vaccines utilising gene transfer or any other immunotherapeutic agent through GMB, UOB, and LTIB affiliated trials at the National Cancer Institute, as well as subjects at extramural sites receiving these agents as part of a multisite trial. These studies include (but are not limited to): 00-C-0137, 00-C-0154, 02-C-0218, 03-C-0176, 04-C-0167, 04-C-0246, 05-C-0017, 05-C-0167, 05-C-0229, 07-C-0106, 07-C-0107, 07-C-0188, 08-C-0166, 09-C-0101, 11-C-0225,12-C-0056, 13-C-0146, 13-C-0153, 13-C-0095,14-C-0142, 14-C-0112,15-C-0205 11-C-0247, 11-C-0262, 13-C-0063, 14-C-0090, 15-C-0012, 15-C-0118, 15-C-0145, 15-C-0178, 15-C-0179, 16-C-0035, 16-C-0048, 16-C-0079, 17-C-0007, 17-C-0023, 17-C-0038, 17-C-0057, and 17-C-0061 • Age: > 18 years

NCT00451022 *(Continued)*

Participants	750 people previously participating in gene transfer or other immunotherapy studies at the National Cancer Institute or extramural sites receiving therapeutic agents as part of a multisite trial
Interventions	Poxviral vectors (vaccinia or fowlpox, or both)
Outcomes	Primary outcome • Annual history and physical examinations for up to 15 years
Starting date	13 September 2004
Contact information	Jennifer L Marte; martej@mail.nih.gov
Notes	Status: recruiting Last update: 23 January 2023

NCT01071941

Study name	rRp450-Phase I trial in liver metastases and primary liver tumours
Methods	Clinical trial, open label Inclusion criteria • Aged 18 years and able to understand and sign a written informed consent form • Histologically confirmed diagnosis of cancer with liver metastases, or histologically confirmed primary liver cancer (e.g. HCC, cholangiocarcinoma, or gallbladder carcinoma). Subjects may have extrahepatic spread of malignancy, except brain metastases. Subjects with a history of > 1 invasive malignancy remain eligible for this study, but in these instances, a liver biopsy is required to document the histology of the liver tumour. An exception is basal cell carcinoma. • Subjects must have primary or metastatic liver malignancies which are surgically unresectable, and exhausted all standard therapeutic options • People with HCC must have received sorafenib as 1 of the standard treatment options prior to being enrolled into the study • No liver surgery (including radiofrequency ablation), chemotherapy (including bevacizumab), immunotherapy, or liver radiotherapy within 4 weeks of enrollment. • ECOG performance status 0, 1, or 2 and life expectancy > 12 weeks based on the investigator's clinical judgement • Serum haematology and chemistry test results as outlined in the protocol • Tumour volume occupies < 50% of liver by volume as assessed by CT scan or MRI scan within 4 weeks of treatment • Negative pregnancy test (serum or urine) in premenopausal women • Prior exposure to herpes simplex virus type 1 as determined by blood test
Participants	40 participants with histologically confirmed cancer with liver metastases or histologically confirmed primary liver cancer (e.g. HCC, cholangiocarcinoma, or gallbladder carcinoma)
Interventions	Biological: administration of rRp450 into the hepatic artery Single group assignment; open label
Outcomes	Primary outcomes: at 3 years • Safety and tolerability of rRp450 administered into the hepatic artery as a single dose

NCT01071941 *(Continued)*

	• Safety and tolerability of rRp450 administered into the hepatic artery as 4 doses administered every 1–2 weeks • Dose-limiting toxicities and maximum dose of rRp450 that can be safely administered into the hepatic artery when administered weekly for 4 doses • rRp450 pharmacokinetics and viral shedding Secondary outcomes: at 3 years • Clinical toxicity • rRp450 replication, tumour response and immune cell infiltrates • Radiographic and pathological assessments of tumour response
Starting date	19 February 2010
Contact information	Kenneth K Tanabe; Massachusetts General Hospital, Boston, Massachusetts, USA
Notes	Status: recruiting Last update: 4 September 2020 Estimated study completion date: July 2023

NCT01869088

Study name	Phase III TACE plus recombinant human adenovirus type 5 Injection for unresectable hepatocellular carcinoma

Methods	Phase 3 clinical trial
	
	Inclusion criteria
	
	• People newly diagnosed as HCC according to European Association for Study of the Liver criteria
	• BCLC stage A or B
	• Child-Pugh class A or B (Child-Pugh score 7)
	• ECOG performance status of 0
	• Patients must have ≥ 1 tumour lesion that meets both of the following criteria
	  ◦ The lesion can be accurately measured in ≥ 1 dimension according to RECIST criteria
	  ◦ The lesion has not been previously treated with surgery, radiotherapy, radiofrequency ablation, percutaneous ethanol or acetic acid injection, or cryoablation
	• Patients who have received previous local therapy treatments (radiofrequency ablation, percutaneous ethanol injection, cryoablation, surgery, resection) to non-target lesions are eligible
	• Local therapy must have been completed ≥ 4 weeks prior to baseline scan
	• Haematology: absolute neutrophil count > 1 × 10^9/L, platelet count > 40 × 10^9/L, Haemoglobin > 9 g/dL (may be transfused to maintain or exceed this level), prothrombin time international normalised ratio < 1.5
	• Biochemistry: total bilirubin < 2 mg/dL, serum creatinine < 1.5 × the upper limit of normal
	• Ability to understand the protocol and to agree to and sign a written informed consent document
	
	Exclusion criteria
	
	• Tumour factors: presence of extrahepatic metastasis, predominantly infiltrative lesion, diffuse tumour morphology with extensive lesions involving both lobes.
	• Vascular complications: hepatic artery thrombosis, partial or complete thrombosis of the main portal vein, tumour invasion of portal branch of contralateral lobe, hepatic vein tumour thrombus, or significant arterioportal shunt not amenable to shunt blockage

NCT01869088 *(Continued)*

	• Liver function: advanced liver disease: ascites, hepatic encephalopathy; with clinically significant gastrointestinal bleeding within the 30 days prior to study entry • Others: renal failure requiring haemo- or peritoneal dialysis; pregnant or lactating women; active sepsis or bleeding; hypersensitivity to intravenous contrast agents; received prior treatment for HCC target lesion. • History of cardiac disease: congestive heart failure > New York Heart Association class 2; active coronary artery disease; cardiac arrhythmias requiring anti-arrhythmic therapy other than beta-blockers or digoxin; hypertension defined as systolic blood pressure > 150 mmHg or diastolic pressure > 90 mmHg despite optimal medical management • Serious non-healing wounds (including wounds healing by secondary intention), acute or non-healing ulcers, or bone fractures within 3 months • Person is, in the opinion of the investigator, unable or unwilling (or both) to comply with treatment and study instructions • Substance abuse (current), psychological, or social conditions that may interfere with the patient's participation in the study or evaluation of the study results • Any active clinically serious infections (> grade 2 National Cancer Institute Common Terminology Criteria for Adverse Events Version 3.0) • HIV infection or AIDS-related illness or serious acute or chronic illness (based on medical history)
Participants	266 participants Age: 18–70 years Gender: all
Interventions	Intervention: recombinant human adenovirus type 5 Injection Active comparator: TACE only Experimental: TACE plus adenovirus
Outcomes	• Overall survival time at 3 years • Number of adverse events at 30 days • Tumour response at 12 weeks
Starting date	January 2013
Contact information	Shi Ming; Sun Yat-sen University, Guangzhou, China
Notes	Status: not yet recruiting Last update: 16 March 2017 Estimated study completion date: January 2018

NCT02395250

Study name	Anti-GPC3 CAR T for treating patients with advanced HCC
Methods	Interventional (clinical trial), open label; single-group assignment
Participants	13 adults and older adults with pathologically confirmed advanced HCC; aged 18–70 years
Interventions	anti-GPC3 CAR T (CAR T cells redirected to Glypican-3 in cancer cells)
Outcomes	• Adverse events attributed to the administration of the anti-GPC3 CAR T cells at 2 years

NCT02395250 *(Continued)*

Starting date	23 March 2015
Contact information	Bo Zhai, MD; RenJi Hospital; Shanghai Cancer Institute Xuhui, Shanghai, China
Notes	Status: completed, but no publication related to the trial Last updated: 28 August 2019 An e-mail request was sent for updated data; a reply from the investigator not yet received

NCT02418988

Study name	NCT02418988
Methods	Interventional (clinical trial): randomised parallel assignment, multicenter, open-label, controlled phase II study
Participants	120 with histopathologically diagnosed HCC; aged 18–80 years
Interventions	TACE plus rAd-p53 vs TACE alone
Outcomes	• Overall survival measured every 6 weeks from starting study treatment to death or 2 years later • Safety as assessed by adverse events • Progression-free survival
Starting date	July 2014
Contact information	Xi An, Shanxi, China, 710032 Xinming Zhou, MD; zhouxmm@fmmu.edu.cn Scott Gao, PhD, MD; scottgao1110@gmail.com
Notes	Status: recruiting Last updated: April 2015 An e-mail request for updated data sent; a reply from the investigator not yet received.

NCT02509169

Study name	Transcatheter arterial embolization combined with p53 gene therapy for treatment of advanced hepatocellular carcinoma
Methods	Phase II clinical trial, randomised, parallel assignment, open label Inclusion criteria • Histopathologically diagnosed unresectable HCC • Aged > 18 years • ECOG 0–2 • BCLC Stage B or C • Child-Pugh score A or B • Normal tests of haemogram, blood coagulation, liver and kidney function

NCT02509169 *(Continued)*

	• Signed the informed consent form Exclusion criteria • Hypersensitive to study drug • Abnormal coagulation condition or bleeding disorder • Infections • Serious conditions which prevent using the study treatment • Pregnant or lactating
Participants	60 Age: 18–85 years
Interventions	TACE plus P53 gene vs TACE
Outcomes	Primary outcome • Overall survival at 2 years Secondary outcomes • Immuno-reaction (lymphocyte counts and subgroup ratios) assessed every week until 3 months after the first treatment • Progression-free survival at 2 years
Starting date	October 2014
Contact information	Yuewei Zhang; zhangyuewei1121@sina.com Gui Gao; scottgao1110@gmail.com
Notes	Status: recruiting Last update: 27 July 2015 Estimated study completion date: December 2016

NCT02561546

Study name	p53 gene therapy in treatment of diabetes concurrent with hepatocellular carcinoma
Methods	Randomised parallel assignment, open-label phase II study
Participants	40 adults and older adults with diabetes and concurrent HCC
Interventions	Trans-catheter embolisation plus p53 gene therapy vs p53 gene therapy
Outcomes	Relevant to this review • Overall survival at 2 years • Progression-free survival at 2 years
Starting date	December 2015
Contact information	First affiliated hospital in Dalian University, Dalian, Liaoning, China Yuewei Zhang, MD, PhD; zhangyuewei1121@sina.com

NCT02561546 *(Continued)*	
	Gui Gao, MD, PhD; scottgao1110@gmail.com
Notes	Status: recruiting Last updated: December 2017 An e-mail request for updated data sent; a reply from the investigator not yet received

NCT02905188	
Study name	Glypican 3-specific chimeric antigen receptor expressing T cells as immunotherapy for patients with hepatocellular carcinoma
Methods	Phase 1, clinical trial; single group assignment; open label Inclusion criteria • Histology-confirmed HCC which is unresectable, recurrent, or metastatic • BCLC Stage A, B, or C • GPC3-positive HCC • Age ≥ 18 years • Karnofsky score > 60% • Life expectancy > 12 weeks • Child-Pugh-Turcotte score < 8 • Needed informed consent Exclusion criteria • History of hypersensitivity reactions to murine protein-containing products or presence of human antimouse antibody prior to enrolment (only people who have received prior therapy with murine antibodies) • History of liver transplantation • Known HIV positive • Active bacterial, fungal, or viral infection (except hepatitis B or hepatitis C virus infections) • Severe previous toxicity from cyclophosphamide or fludarabine
Participants	People with HCC
Interventions	GLYCAR T cells + fludarabine and cytoxan 5 different dosing schedules ($1 \times 10^7/m^2$; $3 \times 10^7/m^2$; $1 \times 10^8/m^2$; $3 \times 10^8/m^2$; $1 \times 10^9/m^2$) 3–6 participants on each dosing schedule
Outcomes	Primary outcome • Number of participants with dose limiting toxicity at 6 weeks Secondary outcomes • Percent of participants with best response (complete remission or partial remission) at 6 weeks • Median T-cell persistence at 6 weeks
Starting date	28 March 2019
Contact information	Tannaz Armaghany; armaghan@bcm.edu

NCT02905188 *(Continued)*

	Ramy Sweidan; rxsweida@texaschildrens.org
Notes	Status: recruiting Last update: 3 February 2023 Estimated study completion date: October 2036

NCT03313596

Study name	Multicenter RCT of ADV-TK gene therapy improving the outcome of liver transplantation for advanced HCC
Methods	Phase 3 trial, randomised, parallel assignment Inclusion criteria • Men and women aged 18–65 years • Clinical diagnosis of advanced primary HCC who could accept liver transplantation • Had unresectable HCC with single tumour diameter > 5 cm and ≤ 10 cm; or numbers of multiple tumours > 3 and ≤ 5, and the total length of foci diameter ≤ 15 cm • Serum AFP ≤ 10,000 ng/mL before liver transplantation • Child-Pugh A-B • No metastasis in extrahepatic main vesicular and extrahepatic lymph node detected during the operation and no metastasis of other organs • Provide written informed consent before screening Exclusion criteria • Metastasis in extrahepatic organs • HCC with invasion in extrahepatic main vesicular and extrahepatic organs • Contraindications of operation of other organ system • Hypersensitivity to adenovirus, ganciclovir, or similar drugs • Serious obstacle of the mechanism of coagulation, haemorrhagic tendency, and abnormal coagulation (≥ 50%) • Plan to accept clinical trials of other antitumour drugs • Immunological deficit • Hepatitis B surface antigen positive and hepatitis B core antibody positive donor • Unsuitable participate assessed by investigator
Participants	180 participants Age: 18–65 years
Interventions	Drug: ADV-TK
Outcomes	Primary outcome • Progression-free survival at 2 years measured from the day of liver transplantation to objective recurrence (MRI or CT) or HCC-related death, whichever occurred first Secondary outcomes • Overall survival measured from the day of liver transplantation to death (time frame 1 year) • Overall survival measured from the day of liver transplantation to death (time frame 2 years) • Time of the tumour progression was the median period from the day of liver transplantation to objective recurrence (MRI or CT) (time frame 2 years)

NCT03313596 *(Continued)*

	• Median overall survival time to 2 years
Starting date	March 2013
Contact information	Danhui Weng; weng.dh@gmail.com
Notes	Status: recruiting Last update: 4 February 2019 Estimated study completion date: December 2019

NCT03680560

Study name	Study of ACTR T cell product in combination with trastuzumab in subjects with HER2-positive advanced solid tumor cancers

Methods	Phase 1 trial, open label
	Inclusion criteria
	• Signed written informed consent obtained prior to study procedures
	• Histologically confirmed HER2-positive advanced solid tumour malignancy with documented disease progression during or immediately following the immediate prior therapy, or within 6 months of completing adjuvant therapy for people with breast cancer
	Participants must have previously received adequate standard therapy for treatment of their malignancy
	• For those with metastatic breast cancer, must have received HER2-directed therapy including trastuzumab, pertuzumab, and ado-trastuzumab in any breast cancer disease setting
	• For those with advanced gastric cancer, adequate prior treatment with HER2-directed chemotherapy is required
	• ≥ 1 measurable lesion by iRECIST
	• Able to provide fresh tumour biopsy or archived block specimen taken since time of most recent anti-HER2 mAb-directed therapy
	• ECOG 0 or 1
	• Life expectancy ≥ 6 months
	• Left ventricular ejection fraction ≥ 50% by multigated acquisition scan or echocardiogram
	• Absolute neutrophil count ≥ 1500/µL
	• Platelet count ≥ 100,000/µL
	• Haemoglobin ≥ 9 g/dL
	• Estimated glomerular filtration rate > 30 mL/minute/1.73 m^2
	Exclusion criteria
	• Glioblastoma multiforme or other primary CNS tumours
	• Clinically significant cardiac disease
	• Clinically significant active infection
	• Clinical history, prior diagnosis, or overt evidence of autoimmune disease
	• Current use of > 5 mg/day of prednisone (or an equivalent glucocorticoid)
	• Prior treatment
	  ◦ Prior cumulative doxorubicin dose ≥ 300 mg/m^2 or equivalent
	  ◦ Chemotherapy within 2 weeks of enrollment
	  ◦ External beam radiation within 2 weeks of enrollment (28 days if central nervous system-directed therapy)

NCT03680560 *(Continued)*

	○ Any monoclonal antibody or other protein therapeutic containing Fc-domains within 4 weeks of enrollment ○ Pertuzumab within 4 months of enrollment ○ Experimental agents within 3 half-lives or 28 days prior to enrollment, whichever is shorter ○ Allogeneic haematopoietic stem cell transplant ○ Prior infusion of a genetically modified therapy ○ Pregnant or breastfeeding
Participants	Age: 18–75 years
Interventions	Biological: ACTR T cell product Drug: trastuzumab
Outcomes	Primary outcomes • Safety and tolerability of ACTR T cell product with trastuzumab as assessed by committee review of dose-limiting toxicities, incidence and severity of adverse events and clinically significant abnormalities of laboratory values (time frame: 42 days) • Determination of recommended phase 2 dose regimen (time frame: 42 days) • Review of dose-limiting toxicities, maximum tolerated dose, incidence and severity of AEs and clinically significant abnormalities of laboratory values Secondary outcomes • Antitumour activity measured at 52 weeks by ○ Overall response rate per iRECIST ○ Best overall response ○ Duration of response ○ Progression-free survival ○ Overall survival • Assessment of persistence of ACTR measured at 52 weeks by ○ Flow cytometry ○ Quantitative polymerase chain reaction • Assessment of ACTR phenotype and function measured by flow cytometry at 52 weeks • Assessment of induction of inflammatory markers and cytokines/chemokines after ACTR T cell product administration at 52 weeks • Levels of inflammatory markers, cytokines/chemokines in blood at 52 weeks • Trastuzumab pharmacokinetics at 52 weeks
Starting date	13 March 2019
Contact information	Glen Weiss, MD Cogent Biosciences, Inc
Notes	Status: terminated Last update: 31 March 2020

NCT04715191

Study name	Interleukin-15 and -21 armored glypican-3-specific chimeric antigen receptor expressed in T Cells for pediatric solid tumors
Methods	Clinical trial, single group assignment, open label

NCT04715191 *(Continued)*

	Inclusion criteria • Relapsed or refractory GPC3-positive solid tumours (as determined by immunohistochemistry with an extent score ≥ Grade 2 (> 25% positive tumour cells) and an intensity score ≥ 2 (scale 0–4)) • Age ≥ 1 year to ≤ 21 years • Lansky or Karnofsky score ≥ 60% • Life expectancy ≥ 16 weeks • BCLC Stage A, B, or C (for participants with HCC only) • Child-Pugh-Turcotte score < 7 (for participants with HCC only) • Obtained informed consent
Participants	24 participants Age: 1–21 years
Interventions	CARE T cells 3 different dosing schedules (1×10^8/m2, $3 \times 10^8/m^2$, $1 \times 10^9/m^2$)
Outcomes	Primary outcome • Number of participants with dose-limiting toxicity at 4 weeks Secondary outcomes • Percent of participants with best response as either complete remission or partial remission at 4 weeks • Median T-cell persistence at 15 years
Starting date	July 2022
Contact information	Andras Heczey; axheczey@txch.org David Steffin; dhsteffi@texaschildrens.org
Notes	Status: not yet recruiting Last update: 20 January 2021 Estimated study completion date: July 2040

ACTR: antibody-coupled T cell receptor; AFP: alpha-fetoprotein; BCLC: Barcelona Clinic Liver Cancer; CT: computed tomography; ECOG: Eastern Cooperative Oncology Group; HCC: hepatocellular carcinoma; MRI: magnetic resonance imaging; TACE: transarterial chemoembolisation; TNM: tumour (T), extent of spread to the lymph nodes (N), and presence of metastasis (M).

RISK OF BIAS

Legend: Low risk of bias | High risk of bias | Some concerns

Risk of bias for analysis 1.1 Overall survival at censored observation (measured at end of treatment, i.e. 20 months)

	Bias					
Study	**Randomisation process**	**Deviations from intended interventions**	**Missing outcome data**	**Measurement of the outcome**	**Selection of the reported results**	**Overall**
Moehler 2019	~	✓	✗	✓	✓	✗

Risk of bias for analysis 1.2 Serious adverse events (Intention-to-treat) (measured at end of treatment, i.e. 20 months)

	Bias					
Study	**Randomisation process**	**Deviations from intended interventions**	**Missing outcome data**	**Measurement of the outcome**	**Selection of the reported results**	**Overall**
Moehler 2019	~	✓	✗	✓	✓	✗

Risk of bias for analysis 1.3 Disease progression (measured at end of treatment, i.e. 20 months)

	Bias					
Study	**Randomisation process**	**Deviations from intended interventions**	**Missing outcome data**	**Measurement of the outcome**	**Selection of the reported results**	**Overall**
Moehler 2019	~	✓	✗	✓	✓	✗

Risk of bias for analysis 1.4 Any adverse events considered non-serious (number of participants) (during 20 months of treatment)

	Bias					
Study	**Randomisation process**	**Deviations from intended interventions**	**Missing outcome data**	**Measurement of the outcome**	**Selection of the reported results**	**Overall**
Moehler 2019	~	✓	✗	✓	✓	✗

Risk of bias for analysis 2.1 All-cause mortality (measured at 1-year follow-up)

	Bias					
Study	**Randomisation process**	**Deviations from intended interventions**	**Missing outcome data**	**Measurement of the outcome**	**Selection of the reported results**	**Overall**
Li 2007	~	~	✓	✓	~	~

Risk of bias for analysis 2.2 All-cause mortality (measured at 2-year follow-up)

	Bias					
Study	**Randomisation process**	**Deviations from intended interventions**	**Missing outcome data**	**Measurement of the outcome**	**Selection of the reported results**	**Overall**
Li 2007	~	~	✓	✓	✓	~

Risk of bias for analysis 2.3 Any adverse events considered non-serious (number of participants)

	Bias					
Study	**Randomisation process**	**Deviations from intended interventions**	**Missing outcome data**	**Measurement of the outcome**	**Selection of the reported results**	**Overall**
Li 2007	~	~	✓	✓	~	~

Risk of bias for analysis 3.1 All-cause mortality (measured at 1-year follow-up)

	Bias					
Study	**Randomisation process**	**Deviations from intended interventions**	**Missing outcome data**	**Measurement of the outcome**	**Selection of the reported results**	**Overall**
Zhu 2018	✓	✓	✓	✓	✓	✓

Risk of bias for analysis 3.2 All-cause mortality (measured at 3-year follow-up)

	Bias					
Study	**Randomisation process**	**Deviations from intended interventions**	**Missing outcome data**	**Measurement of the outcome**	**Selection of the reported results**	**Overall**
Zhu 2018	✓	✓	✓	✓	✓	✓

Risk of bias for analysis 3.3 All-cause mortality (measured at 5-year follow-up) (primary time point)

	Bias					
Study	**Randomisation process**	**Deviations from intended interventions**	**Missing outcome data**	**Measurement of the outcome**	**Selection of the reported results**	**Overall**
Zhu 2018	✓	✓	✓	✓	✓	✓

Risk of bias for analysis 4.1 Overall survival (measured at 6 months)

	Bias					
Study	**Randomisation process**	**Deviations from intended interventions**	**Missing outcome data**	**Measurement of the outcome**	**Selection of the reported results**	**Overall**
Chen 2014	✓	~	✓	✓	~	~

Risk of bias for analysis 4.2 Overall survival (measured at 12 months)

	Bias					
Study	**Randomisation process**	**Deviations from intended interventions**	**Missing outcome data**	**Measurement of the outcome**	**Selection of the reported results**	**Overall**
Chen 2014	✓	~	✓	✓	~	~

Risk of bias for analysis 4.3 Disease progression (measured at 6 months)

	Bias					
Study	**Randomisation process**	**Deviations from intended interventions**	**Missing outcome data**	**Measurement of the outcome**	**Selection of the reported results**	**Overall**
Chen 2014	✓	~	✓	✓	~	~

Risk of bias for analysis 5.1 Disease progression at median 12.8 months

	Bias					
Study	**Randomisation process**	**Deviations from intended interventions**	**Missing outcome data**	**Measurement of the outcome**	**Selection of the reported results**	**Overall**
Tian 2009	~	~	✓	✓	✓	~

Risk of bias for analysis 6.1 Disease progression at 2 weeks

	Bias					
Study	**Randomisation process**	**Deviations from intended interventions**	**Missing outcome data**	**Measurement of the outcome**	**Selection of the reported results**	**Overall**
Habib 2002	~	~	✓	✓	~	~

Risk of bias for analysis 6.2 Any adverse events considered non-serious (number of events)

	Bias					
Study	**Randomisation process**	**Deviations from intended interventions**	**Missing outcome data**	**Measurement of the outcome**	**Selection of the reported results**	**Overall**
Habib 2002	~	~	✓	✓	~	~

Risk of bias for analysis 6.3 Proportion of people without improvement in liver function tests at 2 weeks

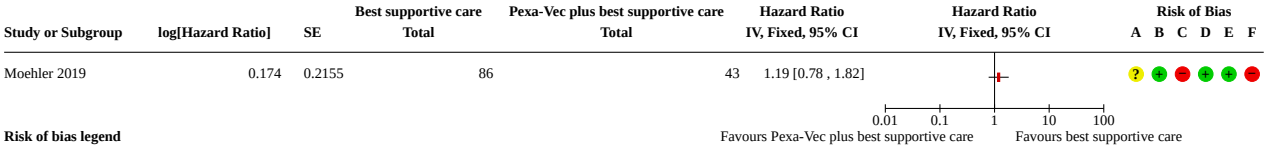
	Bias					
Study	**Randomisation process**	**Deviations from intended interventions**	**Missing outcome data**	**Measurement of the outcome**	**Selection of the reported results**	**Overall**
Habib 2002	~	~	✓	✓	~	~

DATA AND ANALYSES

Comparison 1. Pexastimogene devacirepvec (Pexa-Vec) plus best supportive care versus best supportive care alone

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1.1 Overall survival at censored observation (measured at end of treatment, i.e. 20 months)	1		Hazard Ratio (IV, Fixed, 95% CI)	Totals not selected
1.2 Serious adverse events (Intention-to-treat) (measured at end of treatment, i.e. 20 months)	1		Risk Ratio (M-H, Fixed, 95% CI)	Totals not selected
1.3 Disease progression (measured at end of treatment, i.e. 20 months)	1		Risk Ratio (M-H, Fixed, 95% CI)	Totals not selected
1.4 Any adverse events considered non-serious (number of participants) (during 20 months of treatment)	1		Risk Ratio (M-H, Fixed, 95% CI)	Totals not selected

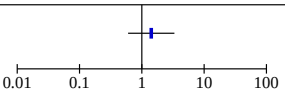
Analysis 1.1. Comparison 1: Pexastimogene devacirepvec (Pexa-Vec) plus best supportive care versus best supportive care alone, Outcome 1: Overall survival at censored observation (measured at end of treatment, i.e. 20 months)



Risk of bias legend
(A) Bias arising from the randomization process
(B) Bias due to deviations from intended interventions
(C) Bias due to missing outcome data
(D) Bias in measurement of the outcome
(E) Bias in selection of the reported result
(F) Overall bias

Analysis 1.2. Comparison 1: Pexastimogene devacirepvec (Pexa-Vec) plus best supportive care versus best supportive care alone, Outcome 2: Serious adverse events (Intention-to-treat) (measured at end of treatment, i.e. 20 months)

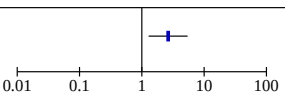
Study or Subgroup	Pexa-Vec plus best supportive care Events	Total	Best supportive care Events	Total	Risk Ratio M-H, Fixed, 95% CI	Risk Ratio M-H, Fixed, 95% CI	Risk of Bias A B C D E F
Moehler 2019	17	86	6	43	1.42 [0.60 , 3.33]		? + − + + −



Risk of bias legend
(A) Bias arising from the randomization process
(B) Bias due to deviations from intended interventions
(C) Bias due to missing outcome data
(D) Bias in measurement of the outcome
(E) Bias in selection of the reported result
(F) Overall bias

Analysis 1.3. Comparison 1: Pexastimogene devacirepvec (Pexa-Vec) plus best supportive care versus best supportive care alone, Outcome 3: Disease progression (measured at end of treatment, i.e. 20 months)

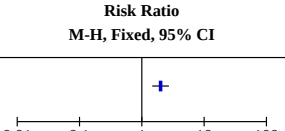
Study or Subgroup	Pexa-Vec plus best supportive care Events	Total	Best supportive care Events	Total	Risk Ratio M-H, Fixed, 95% CI	Risk Ratio M-H, Fixed, 95% CI	Risk of Bias A B C D E F
Moehler 2019	37	86	7	43	2.64 [1.29 , 5.43]		? + − + + −



Risk of bias legend
(A) Bias arising from the randomization process
(B) Bias due to deviations from intended interventions
(C) Bias due to missing outcome data
(D) Bias in measurement of the outcome
(E) Bias in selection of the reported result
(F) Overall bias

Analysis 1.4. Comparison 1: Pexastimogene devacirepvec (Pexa-Vec) plus best supportive care versus best supportive care alone, Outcome 4: Any adverse events considered non-serious (number of participants) (during 20 months of treatment)

Study or Subgroup	Pexa-Vec plus best supportive care Events	Total	Best supportive care Events	Total	Risk Ratio M-H, Fixed, 95% CI	Risk Ratio M-H, Fixed, 95% CI	Risk of Bias A B C D E F
Moehler 2019	84	86	21	43	2.00 [1.47 , 2.72]		? + − + + −



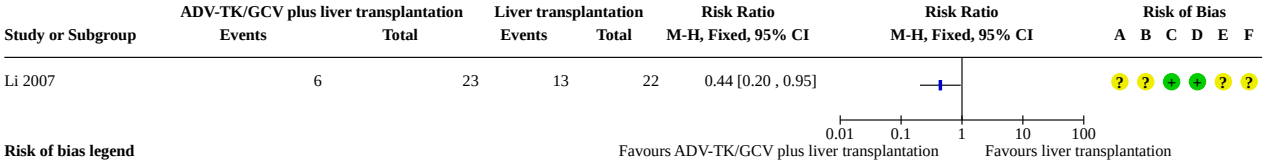
Risk of bias legend
(A) Bias arising from the randomization process
(B) Bias due to deviations from intended interventions
(C) Bias due to missing outcome data
(D) Bias in measurement of the outcome
(E) Bias in selection of the reported result
(F) Overall bias

Comparison 2. Adenovirus-thymidine kinase and ganciclovir (ADV-TK/GCV) plus liver transplantation versus liver transplantation alone

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
2.1 All-cause mortality (measured at 1-year follow-up)	1		Risk Ratio (M-H, Fixed, 95% CI)	Totals not selected

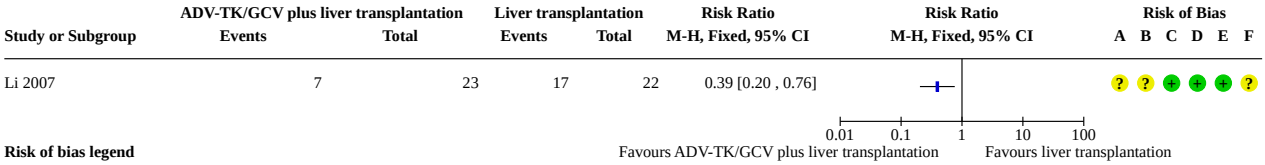
Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
2.2 All-cause mortality (measured at 2-year follow-up)	1		Risk Ratio (M-H, Fixed, 95% CI)	Totals not selected
2.3 Any adverse events considered non-serious (number of participants)	1		Risk Ratio (M-H, Fixed, 95% CI)	Totals not selected

Analysis 2.1. Comparison 2: Adenovirus-thymidine kinase and ganciclovir (ADV-TK/GCV) plus liver transplantation versus liver transplantation alone, Outcome 1: All-cause mortality (measured at 1-year follow-up)



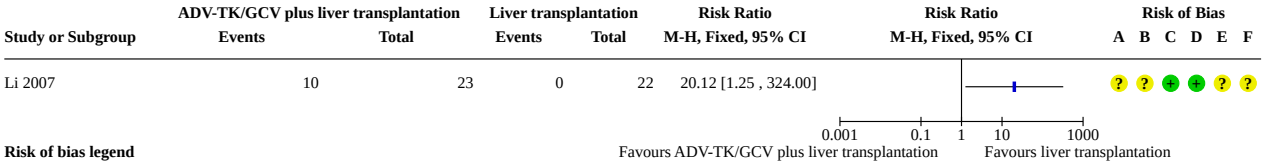
Risk of bias legend
(A) Bias arising from the randomization process
(B) Bias due to deviations from intended interventions
(C) Bias due to missing outcome data
(D) Bias in measurement of the outcome
(E) Bias in selection of the reported result
(F) Overall bias

Analysis 2.2. Comparison 2: Adenovirus-thymidine kinase and ganciclovir (ADV-TK/GCV) plus liver transplantation versus liver transplantation alone, Outcome 2: All-cause mortality (measured at 2-year follow-up)



Risk of bias legend
(A) Bias arising from the randomization process
(B) Bias due to deviations from intended interventions
(C) Bias due to missing outcome data
(D) Bias in measurement of the outcome
(E) Bias in selection of the reported result
(F) Overall bias

Analysis 2.3. Comparison 2: Adenovirus-thymidine kinase and ganciclovir (ADV-TK/GCV) plus liver transplantation versus liver transplantation alone, Outcome 3: Any adverse events considered non-serious (number of participants)

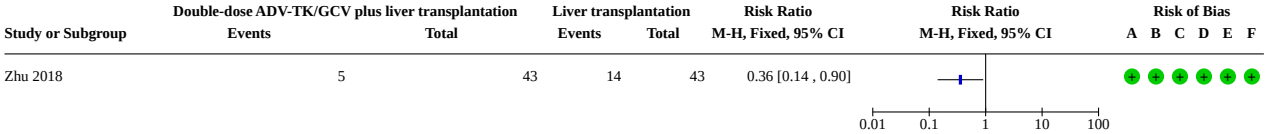


Risk of bias legend
(A) Bias arising from the randomization process
(B) Bias due to deviations from intended interventions
(C) Bias due to missing outcome data
(D) Bias in measurement of the outcome
(E) Bias in selection of the reported result
(F) Overall bias

Comparison 3. Double-dose adenovirus-thymidine kinase and ganciclovir (ADV-TK/GCV) plus liver transplantation versus liver transplantation alone

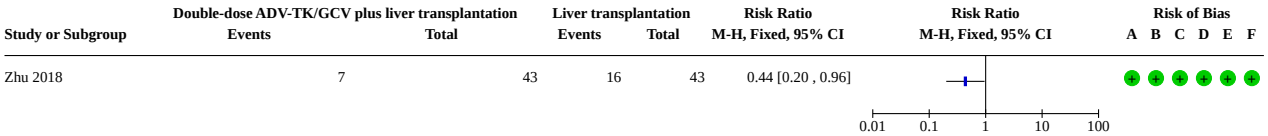
Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
3.1 All-cause mortality (measured at 1-year follow-up)	1		Risk Ratio (M-H, Fixed, 95% CI)	Totals not selected
3.2 All-cause mortality (measured at 3-year follow-up)	1		Risk Ratio (M-H, Fixed, 95% CI)	Totals not selected
3.3 All-cause mortality (measured at 5-year follow-up) (primary time point)	1		Risk Ratio (M-H, Fixed, 95% CI)	Totals not selected

Analysis 3.1. Comparison 3: Double-dose adenovirus-thymidine kinase and ganciclovir (ADV-TK/GCV) plus liver transplantation versus liver transplantation alone, Outcome 1: All-cause mortality (measured at 1-year follow-up)



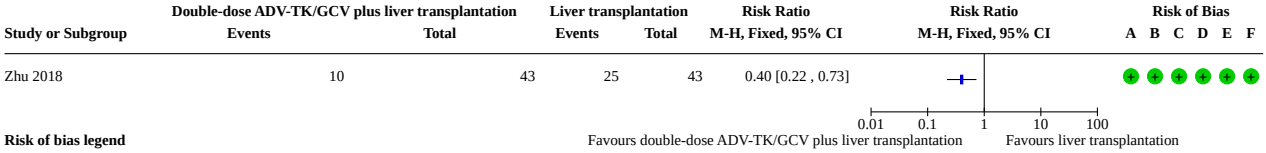
Risk of bias legend
(A) Bias arising from the randomization process
(B) Bias due to deviations from intended interventions
(C) Bias due to missing outcome data
(D) Bias in measurement of the outcome
(E) Bias in selection of the reported result
(F) Overall bias

Analysis 3.2. Comparison 3: Double-dose adenovirus-thymidine kinase and ganciclovir (ADV-TK/GCV) plus liver transplantation versus liver transplantation alone, Outcome 2: All-cause mortality (measured at 3-year follow-up)



Risk of bias legend
(A) Bias arising from the randomization process
(B) Bias due to deviations from intended interventions
(C) Bias due to missing outcome data
(D) Bias in measurement of the outcome
(E) Bias in selection of the reported result
(F) Overall bias

Analysis 3.3. Comparison 3: Double-dose adenovirus-thymidine kinase and ganciclovir (ADV-TK/GCV) plus liver transplantation versus liver transplantation alone, Outcome 3: All-cause mortality (measured at 5-year follow-up) (primary time point)

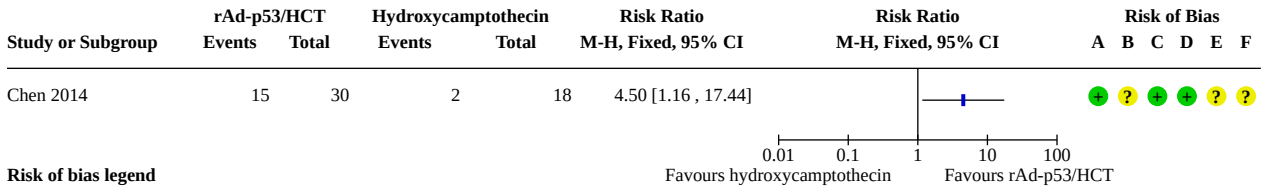


Risk of bias legend
(A) Bias arising from the randomization process
(B) Bias due to deviations from intended interventions
(C) Bias due to missing outcome data
(D) Bias in measurement of the outcome
(E) Bias in selection of the reported result
(F) Overall bias

Comparison 4. Recombinant human adenovirus-p53 with hydroxycamptothecin (rAd-p53/HCT) versus hydroxycamptothecin alone

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
4.1 Overall survival (measured at 6 months)	1		Risk Ratio (M-H, Fixed, 95% CI)	Totals not selected
4.2 Overall survival (measured at 12 months)	1		Risk Ratio (M-H, Fixed, 95% CI)	Totals not selected
4.3 Disease progression (measured at 6 months)	1		Risk Ratio (M-H, Fixed, 95% CI)	Totals not selected

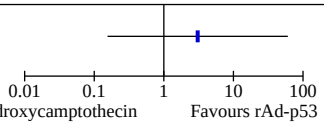
Analysis 4.1. Comparison 4: Recombinant human adenovirus-p53 with hydroxycamptothecin (rAd-p53/HCT) versus hydroxycamptothecin alone, Outcome 1: Overall survival (measured at 6 months)



Risk of bias legend
(A) Bias arising from the randomization process
(B) Bias due to deviations from intended interventions
(C) Bias due to missing outcome data
(D) Bias in measurement of the outcome
(E) Bias in selection of the reported result
(F) Overall bias

Analysis 4.2. Comparison 4: Recombinant human adenovirus-p53 with hydroxycamptothecin (rAd-p53/HCT) versus hydroxycamptothecin alone, Outcome 2: Overall survival (measured at 12 months)

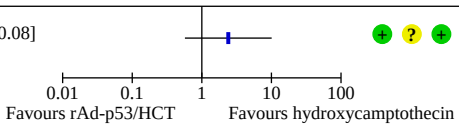
Study or Subgroup	rAd-p53 Events	rAd-p53 Total	Hydroxycamptothecin Events	Hydroxycamptothecin Total	Risk Ratio M-H, Fixed, 95% CI	Risk Ratio M-H, Fixed, 95% CI	Risk of Bias A B C D E F
Chen 2014	2	30	0	18	3.06 [0.16 , 60.47]		+ ? + + ? ?



Risk of bias legend
(A) Bias arising from the randomization process
(B) Bias due to deviations from intended interventions
(C) Bias due to missing outcome data
(D) Bias in measurement of the outcome
(E) Bias in selection of the reported result
(F) Overall bias

Analysis 4.3. Comparison 4: Recombinant human adenovirus-p53 with hydroxycamptothecin (rAd-p53/HCT) versus hydroxycamptothecin alone, Outcome 3: Disease progression (measured at 6 months)

Study or Subgroup	rAd-p53/HCT Events	rAd-p53/HCT Total	Hydroxycamptothecin Events	Hydroxycamptothecin Total	Risk Ratio M-H, Fixed, 95% CI	Risk Ratio M-H, Fixed, 95% CI	Risk of Bias A B C D E F
Chen 2014	8	30	2	18	2.40 [0.57 , 10.08]		+ ? + + ? ?



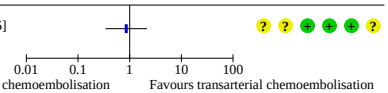
Risk of bias legend
(A) Bias arising from the randomization process
(B) Bias due to deviations from intended interventions
(C) Bias due to missing outcome data
(D) Bias in measurement of the outcome
(E) Bias in selection of the reported result
(F) Overall bias

Comparison 5. Recombinant human adenovirus-p53/5-fluorouracil (rAd-p53/5-Fu) plus transarterial chemoembolisation versus transarterial chemoembolisation alone

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
5.1 Disease progression at median 12.8 months	1		Risk Ratio (M-H, Fixed, 95% CI)	Totals not selected

Analysis 5.1. Comparison 5: Recombinant human adenovirus-p53/5-fluorouracil (rAd-p53/5-Fu) plus transarterial chemoembolisation versus transarterial chemoembolisation alone, Outcome 1: Disease progression at median 12.8 months

Study or Subgroup	rAd-p53/5-FU plus transarterial chemoembolisation Events	rAd-p53/5-FU plus transarterial chemoembolisation Total	Transarterial chemoembolisation Events	Transarterial chemoembolisation Total	Risk Ratio M-H, Fixed, 95% CI	Risk Ratio M-H, Fixed, 95% CI	Risk of Bias A B C D E F
Tian 2009	6	23	7	23	0.86 [0.34 , 2.16]		? ? + + + ?



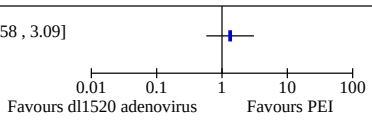
Risk of bias legend
(A) Bias arising from the randomization process
(B) Bias due to deviations from intended interventions
(C) Bias due to missing outcome data
(D) Bias in measurement of the outcome
(E) Bias in selection of the reported result
(F) Overall bias

Comparison 6. E1B-deleted (dl1520) adenovirus versus percutaneous ethanol injection (PEI)

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
6.1 Disease progression at 2 weeks	1		Risk Ratio (M-H, Fixed, 95% CI)	Totals not selected
6.2 Any adverse events considered non-serious (number of events)	1		Risk Ratio (M-H, Fixed, 95% CI)	Totals not selected
6.3 Proportion of people without improvement in liver function tests at 2 weeks	1		Risk Ratio (M-H, Fixed, 95% CI)	Totals not selected

Analysis 6.1. Comparison 6: E1B-deleted (dl1520) adenovirus versus percutaneous ethanol injection (PEI), Outcome 1: Disease progression at 2 weeks

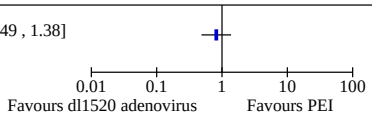
	E1B-deleted (dl1520) adenovirus		PEI		Risk Ratio	Risk Ratio	Risk of Bias
Study or Subgroup	Events	Total	Events	Total	M-H, Fixed, 95% CI	M-H, Fixed, 95% CI	A B C D E F
Habib 2002	4	5	3	5	1.33 [0.58 , 3.09]		? ? + + ? ?



Risk of bias legend
(A) Bias arising from the randomization process
(B) Bias due to deviations from intended interventions
(C) Bias due to missing outcome data
(D) Bias in measurement of the outcome
(E) Bias in selection of the reported result
(F) Overall bias

Analysis 6.2. Comparison 6: E1B-deleted (dl1520) adenovirus versus percutaneous ethanol injection (PEI), Outcome 2: Any adverse events considered non-serious (number of events)

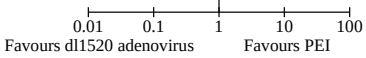
	E1B-deleted (dl1520) adenovirus		PEI		Risk Ratio	Risk Ratio	Risk of Bias
Study or Subgroup	Events	Total	Events	Total	M-H, Fixed, 95% CI	M-H, Fixed, 95% CI	A B C D E F
Habib 2002	4	5	5	5	0.82 [0.49 , 1.38]		? ? + + ? ?



Risk of bias legend
(A) Bias arising from the randomization process
(B) Bias due to deviations from intended interventions
(C) Bias due to missing outcome data
(D) Bias in measurement of the outcome
(E) Bias in selection of the reported result
(F) Overall bias

Analysis 6.3. Comparison 6: E1B-deleted (dl1520) adenovirus versus percutaneous ethanol injection (PEI), Outcome 3: Proportion of people without improvement in liver function tests at 2 weeks

Study or Subgroup	E1B-deleted (dl1520) adenovirus Events	Total	PEI Events	Total	Risk Ratio M-H, Fixed, 95% CI	Risk Ratio M-H, Fixed, 95% CI	Risk of Bias A	B	C	D	E	F
Habib 2002	5	5	4	5	1.22 [0.73 , 2.06]		?	?	+	+	?	?



Risk of bias legend

(A) Bias arising from the randomization process
(B) Bias due to deviations from intended interventions
(C) Bias due to missing outcome data
(D) Bias in measurement of the outcome
(E) Bias in selection of the reported result
(F) Overall bias

ADDITIONAL TABLES

Table 1. Description of the included randomised controlled trials

Study ID (Country)	Protocol registry	Design	Trial phase	No of participants	Males (%)	Mean age (years)	Gene therapy	Other treatment in experimental arm	Overall risk of bias
Chen 2014 (China)	No	Parallel group	NR (seems to be a pilot phase I study)[a]	48	73.3	Range 35–76	rAd-p53	Hydroxycamptothecin 20 mg	Some concerns
Habib 2002 (Egypt)	No	Parallel group	Phase I	10	80.0	Range 46–74	E1B-deleted (dl1520) adenovirus	Percutaneous ethanol injection	Some concerns
Li 2007 (China)	No	Parallel group	NR (seems to be phase II)[b]	45	100	44.3	ADV-TK/GCV	Liver transplantation	Some concerns
Moehler 2019 (multiple)	Yes	Parallel group	Phase IIb	129	72.0	60	Pexa-Vec	Best supportive care	High
Tian 2009 (China)	No	Parallel group	Pilot phase II	46	74.0	55	rAd-p53	Transcatheter arterial chemoembolisation	Some concerns
Zhu 2018 (China)	Yes	Parallel group	NR (seems to be phase II)[c] ClinicalTrials.gov (NCT02202564)	86	37.0	50.4	Double dose ADV-TK/GCV	Liver transplantation	Low

[a]Our study provided an initial report on the clinical application of p53 gene. p.28.
[b]A single-dose intratumoural injection of 5.0×10^{11} viral particles ADV-TK caused an objective response with no significant toxicity (phase I test, Supplementary Data 3). p.5849 (Li 2007).

[c]Described as Phase II in another study (see Chakraborty 2022).

NR: not reported.

Table 2. Toxicity and complications of gene therapy reported in Tian 2009. Recombinant human adenovirus-p53 and 5-fluorouracil (rAd-p53/5-Fu) plus transarterial chemoembolisation (TACE) versus transarterial chemoembolisation

Description	Number of injections	Experimental intervention (rAd-p53/5-Fu and TACE)					Control intervention: TACE alone
		Grade 1 (%)	Grade 2 (%)	Grade 3 (%)	Grade 4 (%)	Total	Total
Nausea	166	22 (13)	8 (5)	0	0	30 (18)	Not reported
Fatigue	166	15 (9)	5 (3)	0	0	20 (12)	
Vomiting	166	5 (3)	2 (1)	0	0	7 (4)	
Leukopenia	166	4 (2)	0	0	0	4 (2)	
Anaemia	166	2 (1)	1 (1)	0	0	3 (2)	

These were non-serious adverse events (Tian 2009).

APPENDICES

Appendix 1. Varieties of gene therapy

Ex vivo gene therapy	In vivo gene therapy
1. Glypican-3 (GPC-3)	1. Non-viral delivery system
2. Alpha-fetoprotein (AFP)	1.1. Nanoparticles (NPs)
3. Cluster of differentiation 147 (CD147)	1.1.1. Nanoparticles used as monotherapy
4. Mucin-1 (Muc1)	in vitro
5. Epithelial cell adhesion molecule	1.1 2. Nanoparticles used for combination
(EpCAM)	therapy in vitro
6. NY-ESO 1	1.2. Virus-like particles (VLPs)
	2. Viral delivery
	2.1. Adeno and adeno-associated virus
	2.2. Vaccinia virus
	2.3. Lentivirus

Ex vivo: outside of the living body; an organ, cells, or tissue is taken from a living body for a treatment or procedure, and then returned to the living body.
In vivo: in the living organism; an experiment done in the body of a living organism.
In vitro: experiment/procedure done outside of the living organism.

Appendix 2. Gene therapy procedures in the included trials

Study	Experimental intervention		Control intervention	
	Name (brand)	Description	Name	Description
Chen 2014	Ad-p53 injection (Gendicine, Shenzhen SiBiono GenTech)	Recombinant adenovirus p53 for injection. The participants in the treatment group were given Gendicine (1012vp (viral particles)) plus hydroxycamptothecin 20 mg, once a week for a course continuously for 3 weeks.	Hydroxycamptothecin (HCT)	Arterial infusion with hydroxycamptothecin 20 mg
Habib 2002	E1B-deleted (dl1520) adenovirus (Sterling, UK)	dl1520 adenovirus for intravenous injection in the arm vein (vena mediana cubiti) for the first dose (day 1), then by direct intratumoural injection under ultrasound guidance on days 2, 15, 16, 29, and 30. Virus vector administration was under local anaesthesia and participants were carefully observed in hospital for 2 hours following the treatment.	Percutaneous ethanol injection (PEI)	Sterile 95% ethanol was administered with an 18- to 20-gauge spinal needle using the Livraghi technique.

(Continued)

		All given doses were 1 mL in volume and contained 3×10^{11} PFU of dl1520. (PFU: plaque forming units)		
Li 2007	Adenovirus-mediated delivery of herpes simplex virus thymidine kinase (ADV-TK injection, Tian Dakang Co)	Herpes simplex virus thymidine kinase converts a benign substance (prodrug) ganciclovir into toxic nucleotide analogues, which are incorporated into DNA during cell division, terminate DNA replication, and lead to cancer cell death.	Liver transplantation	Orthotopic liver transplantation was not described.
Moehler 2019	Pexa-Vec plus best supportive care	A thymidine kinase gene-inactivated oncolytic vaccinia virus engineered to express the transgenes human granulocyte-macrophage colony stimulating factor and β galactosidase.	Best supportive care	Best supportive care was not described.
Tian 2009	Ad-p53 injection (Gendicine, Shenzhen SiBiono GenTech)	A recombinant human serotype 5 adenovirus in which the E1 region is replaced by a human wild-type p53 expression cassette.	Transarterial chemoembolisation (TACE)	Performed by the Seldinger technique and through femoral artery access.
Zhu 2018	Double dose (ADV-TK/GCV, Tian Dakang Co)	Adenovirus (Adv)-mediated delivery of herpes simplex virus thymidine kinase (adv/tk) into tumour cells. TK transduction effectively eradicates cancer cells via a bystander effect that acts on the targeted lesion and distant lesions.	Liver transplantation	Orthotopic liver transplantation and subsequent immunosuppression therapy

Appendix 3. Search strategies

Database	Time span	Search strategy
Cochrane Hepato-Biliary Group Controlled Trials Register (searched via the Cochrane Register of Studies Web)	20 January 2023	(gene* near (therap* or treat* or transfer*)) AND (((liver or hepato*) and (carcinom* or cancer* or neoplasm* or malign* or tumo*)) or HCC)
Cochrane Central Register of Controlled Trials	2023, Issue 1	#1 MeSH descriptor: [Genetic Therapy] explode all trees #2 MeSH descriptor: [Gene Transfer Techniques] explode all trees #3 (gene* near/3 (therap* or treat* or transfer*)) #4 #1 OR #2 OR #3

(Continued)

		#5 MeSH descriptor: [Carcinoma, Hepatocellular] explode all trees #6 MeSH descriptor: [Liver Neoplasms] explode all trees #7 (((liver or hepato*) and (carcinom* or cancer* or neoplasm* or malign* or tumo*)) or HCC) #8 #5 OR #6 OR #7 #9 #4 AND #8
MEDLINE Ovid	1946 to 20 January 2023	1. exp Genetic Therapy/ 2. Gene Transfer Techniques/ 3. (gene* adj3 (therap* or treat* or transfer*)).mp. [mp=title, book 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] 4. 1 or 2 or 3 5. exp Carcinoma, Hepatocellular/ 6. exp Liver Neoplasms/ 7. (((liver or hepato*) and (carcinom* or cancer* or neoplasm* or malign* or tumo*)) or HCC).mp. [mp=title, book 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] 8. 5 or 6 or 7 9. 4 and 8 10. (randomized controlled trial or controlled clinical trial or retracted publication or retraction of publication).pt. 11. clinical trials as topic.sh. 12. (random* or placebo*).ab. or trial.ti. 13. 10 or 11 or 12 14. exp animals/ not humans.sh. 15. 13 not 14 16. 9 and 15
Embase Ovid	1974 to 20 January 2023	1. exp gene therapy/ 2. exp gene transfer/ 3. (gene* adj3 (therap* or treat* or transfer*)).mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword heading word, floating subheading word, candidate term word]

(Continued)

4. 1 or 2 or 3

5. exp liver cell carcinoma/

6. exp liver tumor/

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

8. 5 or 6 or 7

9. 4 and 8

10. Randomized controlled trial/ or Controlled clinical study/ or randomization/ or intermethod comparison/ or double blind procedure/ or human experiment/ or retracted article/

11. (random$ or placebo or parallel group$1 or crossover or cross over or assigned or allocated or volunteer or volunteers).ti,ab.

12. (compare or compared or comparison or trial).ti.

13. ((evaluated or evaluate or evaluating or assessed or assess) and (compare or compared or comparing or comparison)).ab.

14. (open adj label).ti,ab.

15. ((double or single or doubly or singly) adj (blind or blinded or blindly)).ti,ab.

16. ((assign$ or match or matched or allocation) adj5 (alternate or group$1 or intervention$1 or patient$1 or subject$1 or participant$1)).ti,ab.

17. (controlled adj7 (study or design or trial)).ti,ab.

18. (erratum or tombstone).pt. or yes.ne.

19. or/10-18

20. (random$ adj sampl$ adj7 ('cross section$' or questionnaire$ or survey$ or database$1)).ti,ab. not (comparative study/ or controlled study/ or randomi?ed controlled.ti,ab. or randomly assigned.ti,ab.)

21. Cross-sectional study/ not (randomized controlled trial/ or controlled clinical study/ or controlled study/ or randomi?ed controlled.ti,ab. or control group$1.ti,ab.)

22. (((case adj control$) and random$) not randomi?ed controlled).ti,ab.

23. (Systematic review not (trial or study)).ti.

24. (nonrandom$ not random$).ti,ab.

25. 'Random field$'.ti,ab.

26. (random cluster adj3 sampl$).ti,ab.

27. (review.ab. and review.pt.) not trial.ti.

(Continued)

		28. 'we searched'.ab. and (review.ti. or review.pt.) 29. 'update review'.ab. 30. (databases adj4 searched).ab. 31. (rat or rats or mouse or mice or swine or porcine or murine or sheep or lambs or pigs or piglets or rabbit or rabbits or cat or cats or dog or dogs or cattle or bovine or monkey or monkeys or trout or marmoset$1).ti. and animal experiment/ 32. Animal experiment/ not (human experiment/ or human/) 33. or/20-32 34. 19 not 33 35. 9 and 34
LILACS (VHL Regional Portal)	1982 to 20 January 2023	((gene* AND (therap* OR treat* OR transfer*))) AND ((((liver OR hepato*) AND (carcinom* OR cancer* OR neoplasm* OR malign* OR tumo*)) OR hcc)) AND (db:("LILACS"))
Science Citation Index Expanded and Conference Proceedings Citation Index – Science (Web of Science)	1900 to 20 January 2023	#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=(gene* near (therap* or treat* or transfer*))

Appendix 4. Gene therapy for people with hepatocellular carcinoma (review): domain-based assessment with the RoB 2 tool (consensus between the two review authors)

Summary RoB 2 by two review authors is available at

figshare.com/articles/journal_contribution/Risk_of_bias_judgements_and_support_for_judgements_xls/25828471

Detailed risk of bias assessment using RoB 2 tool by two review authors is available at

figshare.com/articles/journal_contribution/Domain-based_assessment_with_the_RoB_2_tool_consensus/25848664

Appendix 5. Adverse events reported in a non-randomised study

Non-randomised study reporting adverse events

Author	Study design	Sample size	Intervention	Manufacturer	Research question	Treatment-related adverse effects					
						Frequency (%)					
						Fever	Transient abdominal pain	Nausea and vomiting	Cholecystitis	Thrombocytopenia	Leukopenia
Liu 2015	Retrospective observational	15 participants with hepatocellular carcinoma with Barcelona clinic liver cancer (BCLC) stage B	p53 gene (rAd-p53) (Gendicine) plus transarterial chemoembolisation (TACE)	Gendicine from Shenzhen Sibiono Genetech Co Ltd, China	Safety	15 (100)	7 (46.7)	5 (33.3)	4 (26.7)	3 (20)	3 (20)

WHAT'S NEW

Date	Event	Description
16 July 2024	Amended	Plain language summary adjusted in response to feedback from translators.

HISTORY

Protocol first published: Issue 9, 2020
Review first published: Issue 6, 2024

CONTRIBUTIONS OF AUTHORS

CN: collected data, entered data, assessed risk of bias, analysed and interpreted, assisted with certainty of the evidence, wrote the review with suggestions of team members.

HN: assisted with data synthesis, assessed risk of bias, rated certainty of the evidence, interpreted results, commented on the review.

HHA: collected data, assisted with bias risk assessment, assisted with data synthesis, interpreted results, commented on the review.

NHH: assisted with background description, assisted with bias risk assessment, commented on the review.

DN: assisted with data extraction, assisted with bias risk assessment, interpreted results, assisted with writing the review, commented on the review.

All authors approved the review for publication.

Text in this review may overlap with other Cochrane review protocols and Cochrane reviews. This is because all protocols and reviews follow the Cochrane methodology.

DECLARATIONS OF INTEREST

CN: none.

HN is a Cochrane Editor, but had no role in the editorial process for this review.

HHA: none.

NHH: none.

DN is the Managing Editor of the Cochrane hepato-Biliary Group, but had no role in the editorial process for this review.

SOURCES OF SUPPORT

Internal sources

- James Cook University, Queensland, Australia

  Support to CN
- Newcastle University Medicine, Johor, Malaysia

  Support to HN
- School of Medicine, IMU University, Kuala Lumpur, Malaysia

  Support to HHA, NHH
- Hepato-Biliary Group, the Copenhagen Trial Unit, Centre for Clinical Intervention Research, The Capital Region, Copenhagen University Hospital — Rigshospitalet, Denmark

  Support to Dimitrinka Nikolova

External sources

- The authors declare that no funding was received for this systematic review, Australia

  None of the authors received financial support for this review

DIFFERENCES BETWEEN PROTOCOL AND REVIEW

We made the objective more explicit; that is, "To evaluate the benefits and harms of gene therapy in people with hepatocellular carcinoma, irrespective of sex, administered dose, and type of formulation."

Instead of the number of participants with an adverse event, we reported data on number of events found in two included trials (Habib 2002; Tian 2009).

We added some further text as to what we will do in the future if more trials are identified and for any reason, we cannot analyse the data using the intention-to-treat (ITT) principle and cannot assess the percentage of dropouts for each included trial or other information of relevance to the analysis (Dealing with missing data).

INDEX TERMS

Medical Subject Headings (MeSH)

Bias; *Carcinoma, Hepatocellular [genetics] [mortality] [therapy]; Cause of Death; *Genetic Therapy [methods]; *Liver Neoplasms [genetics] [mortality] [therapy]; Quality of Life; *Randomized Controlled Trials as Topic

MeSH check words

Female; Humans; Male; Middle Aged