NATIONAL INSTITUTE FOR HEALTH AND CLINICAL EXCELLENCE

INTERVENTIONAL PROCEDURES PROGRAMME

Interventional procedure overview of cryotherapy for the treatment of liver metastases

Cancer cells can spread from where they start (the 'primary tumour') to other parts of the body to form one or more 'secondary tumours', which are also known as 'metastases'. Liver metastases occur when cancer spreads from other parts of the body to the liver.

Cryotherapy is a process that uses cold temperatures to destroy cancer cells; it is applied directly to the tumour through a special needle.

Introduction

The National Institute for Health and Clinical Excellence (NICE) has prepared this overview to help members of the Interventional Procedures Advisory Committee (IPAC) make recommendations about the safety and efficacy of an interventional procedure. It is based on a rapid review of the medical literature and specialist opinion. It should not be regarded as a definitive assessment of the procedure.

Date prepared

This overview was prepared in April 2010

Procedure name

- Cryotherapy for the treatment of liver metastases

Specialty societies

- British Society of Interventional Radiology
- British Society of Gastrointestinal and Abdominal Radiology
- Association of Upper Gastrointestinal Surgeons of Great Britain and Ireland.

Description

Indications and current treatment

Liver metastases are most commonly due to the spread of colorectal cancer, but they may also result from other malignancies, such as lung and gastric cancer.

The number, location and size of the metastases are the key determinants of treatment intent as well as of treatment choice. For a minority of patients, surgical resection with curative intent may be possible. For most patients however, treatment intent is palliative. Options for palliative treatment include systemic chemotherapy, external beam radiotherapy, other thermal ablation techniques (such as radiofrequency or microwave ablation), and selective internal radiation therapy. Multiple treatment modalities may be used for individual patients.

Thermal ablation therapy procedures including cryotherapy ablation may be options as the primary treatment for liver metastases, if the patient is not considered suitable for surgery or in the treatment of post-resection recurrence. They may also be used as an adjunct to hepatic resection to ablate small-volume disease in the future remnant liver.

What the procedure involves

Cryotherapy for liver metastases aims to allow treatment of lesions that are not suitable for surgical resection, with minimal morbidity.

Cryotherapy can be delivered as part of an open resection procedure, or percutaneously. If part of an open resection procedure it is usually performed with the patient under general anaesthesia, however, the percutaneous approach can be used with the patient under local anaesthesia (although general anaesthesia is sometimes used). In open operations the procedure is performed under direct vision or with the assistance of intra-operative ultrasound. Percutaneous procedures require imaging guidance with ultrasound, computed tomography or magnetic resonance imaging.

Once the lesion is located, 1 or more probes are inserted into the tumour (multiple probes are used for larger lesions). The probe(s) delivers a coolant at subfreezing temperatures at the tip of the probe. An ice ball is created around the tip of the probe, destroying cells through direct freezing, dehydration and hypoxia. Each freeze cycle is followed by a thaw cycle. A double freeze-thaw cycle is usually performed to ablate the tumour, with the aim of extending the ice ball approximately 1 cm beyond the tumour margins. Additional freeze-thaw cycles may be repeated if necessary. Once the cycles have been completed the probe(s) are removed.

The maximum recommended hepatic tumour size for cryotherapy is around 4.0 cm. Lesions of such size or smaller can be treated with a single probe,

which causes less morbidity than multiple probe use. A number of devices are available for this procedure; newer percutaneous probes tend to be narrower.

Literature review

Rapid review of literature

The medical literature was searched to identify studies and reviews relevant to cryotherapy for the treatment of liver metastases. Searches were conducted of the following databases, covering the period from their commencement to 26 January 2010, and updated to 2 August 2010: MEDLINE, PREMEDLINE, EMBASE, Cochrane Library and other databases. Trial registries and the Internet were also searched. No language restriction was applied to the searches (see appendix C for details of search strategy). Relevant published studies identified during consultation or resolution that are published after this date may also be considered for inclusion.

The following selection criteria (table 1) were applied to the abstracts identified by the literature search. Where selection criteria could not be determined from the abstracts the full paper was retrieved.

Table 1 Inclusion criteria for identification of relevant studies

Characteristic	Criteria
Publication type	Clinical studies were included. Emphasis was placed on identifying good quality studies. Abstracts were excluded where no clinical outcomes were reported, or where the paper was a review, editorial, or a laboratory or animal study. Conference abstracts were also excluded because of the difficulty of appraising study methodology, unless they reported specific adverse events that were not available in the published literature.
Patient	Patients with liver metastases
Intervention/test	Cryotherapy (all approaches)
Outcome	Articles were retrieved if the abstract contained information relevant to the safety and/or efficacy.
Language	Non-English-language articles were excluded unless they were thought to add substantively to the English-language evidence base.

List of studies included in the overview

This overview is based on 1238 patients from 1 randomised controlled trial (RCT)[1], 2 non-randomised controlled studies[23], 2 case series[45], and 1 case report[6].

Other studies that were considered to be relevant to the procedure but were not included in the main extraction table (table 2) have been listed in appendix A.

Table 2 Summary of key efficacy and safety findings on cryotherapy for the treatment of liver metastases

Abbreviations used: CEA, carcinoembryonic antigen; Cryo, cryotherapy; CT, computed tomography; GI, Gastrointestinal; MRI, magnetic resonance imaging; NR, not reported;

Study details	Key efficacy findings	Key safety findings	Comments
Korpan N N (1997)[1] **RCT** Ukraine Recruitment period: 1983 to 1992 Study population: Hepatic metastases from a variety of primaries (65 to 68% colorectal primaries). One metastasis n = 37, 2 n = 46, 3 or more n = 40. **n = 123 (63 cryo)** Age: 41 years (mean) Sex: 71% Male Patient selection criteria: isolated primary manifestation of solitary liver metastases, or multiple nodules in 1 or both lobes of the liver. Technique: liver mobilised at laparotomy. All types and/or approaches to deliver cryotherapy vs surgical resection only. Plus systemic chemotherapy in both groups. **Follow-up: 5 months to 10 years (range)** Conflict of interest/source of funding: not reported	Number of patients analysed: **123 (63 cryotherapy vs 60 surgical resection)** **Survival** Period / Cryotherapy / Surgical resection 3 years / 60% / 51% 5 years / 44% / 36% 10 years / 19% / 8% Disease-free survival was 14.3% (9/63) in the cryotherapy group and 5.0% (3/60) in the surgical resection group at 10-year follow-up (measurement of significance not reported). **Biochemical outcomes** Decrease of carcinoembryonic antigen level to normal was quicker in patients treated by cryotherapy compared with surgical resection ($p < 0.05$) (absolute figures not reported). **Treatment characteristics** Length of stay was significantly shorter following cryotherapy 7.5 ± 0.5 days, than following surgical resection 8.6 ± 0.4 days (measurement of significance not reported).	**Complications** No mortality reported in either group. Outcome / Cryo / Resection Fever / 38.4 (± 0.7) °C / 38.7 (± 0.6) °C Pain at 5-day follow-up / 83% / 62% pronounced pain at 5-day follow-up / 17% / 9% Severe pain at 5-day follow-up / 0% / 29% Blood transfusion, plasma, or albumin / 25.4% (16/63) / 35.0% (21/60) Pneumonia / 4.8% (3/63) / 3.3% (2/60) Septic wound / 3.2% (2/63) / 1.7% (1/60) Bleeding / 0% (0/63) / 3.3% (2/60) Bile leakage / 0% (0/63) / NR Abdominal infection / 0% (0/63) / NR Subphrenic abscess / NR / 11.7% (7/60) Pleural effusion / NR / 5.0% (3/60) (measurement of significance not reported).	**Follow-up issues**: Loss to follow-up not reported. Length of follow-up for disease free survival analysis not reported except for 10 years. **Study design issues**: Patients assessed at baseline with CT and/or MRI. Method of randomisation and blinding not described. Tumour margin of > 1cm considered to be negative. Postoperative treatment and concomitant therapy were identical for both groups. **Study population issues**: No significant difference between groups in demographic characteristics symptoms, liver metastasis, disease free interval (from primary resection to first liver manifestation. 14 patients in the cryotherapy group were described as having non-resectable liver metastases **Other issues**: Very mixed range of cryotherapy techniques used, all via laparotomy. Surgical techniques may have progressed considerably since the study period

Abbreviations used: CEA, carcinoembryonic antigen; Cryo, cryotherapy; CT, computed tomography; GI, Gastrointestinal; MRI, magnetic resonance imaging; NR, not reported;

Study details	Key efficacy findings	Key safety findings	Comments
Niu R (2007)[2] **Non-randomised controlled trial** Australia Recruitment period: 1990 to 2006 Study population: Patients with colorectal liver metastases. Mean number of lesions = 2.9, mean size of largest lesion = 5cm. **n = 415 (124 cryo)** Age: 62 years (mean) Sex: 58% Male Patient selection criteria: not reported. Technique: surgery with curative intent. Laparotomy through right sub-costal incision. With ultrasound guidance combined surgical resection plus cryotherapy (with a variety of devices) for remaining contra-lobar lesions. For lesions greater than 3 cm in diameter 2 or more probes were used. Vs surgical resection only. Adjuvant hepatic artery chemotherapy for both groups according to protocol. **Follow-up: 25 months (median)** Conflict of interest/source of funding: not reported	Number of patients analysed: **402 (119 cryotherapy vs 283 surgical resection)** **Survival** There was no statistically significant difference in median survival between the cryotherapy group (29 months) (range 1 to 117 months) and the resection group (34 months) (range 1 to 124 months) (p = 0.206). Five factors were independent predictors of overall survival in multivariate analysis. Well or moderately differentiated colorectal cancer (p = 0.029), no extra hepatic disease at baseline (p = 0.026), liver lesions < 4 cm (p = 0.003), postoperative CEA of 5 ng/ml or less (p = 0.005), and no liver recurrence (p = 0.003). .	Safety outcomes were not reported on	**Follow-up issues**: Prospective follow-up. Patients who died during 30-day follow-up were excluded from survival and recurrence analysis. Eight patients in the resection group (2.7%) and 5 in the cryotherapy group (4.0%). No analysis done to compare these patients with those with follow up data. **Study design issues**: Patients assessed by CT scanning at baseline. Tumour margin of > 1cm considered to be negative. Patient selection for treatment modality not reported. **Study population issues**: Lesions were considered unresectable if clear surgical margin and preservation of hepatic parenchyma were not possible. It is not clear whether respectability was used as a criterion for allocation to treatment arm. Patients in the cryotherapy group were more likely to have bilateral disease, a greater number of lesions, and larger lesions (all p < 0.0001). **Other issues**: None

Outcome	Cryotherapy	Surgical resection	p =
Overall recurrence	81.5% (97/119)	67.8% (192/283)	0.005
Liver recurrence	59.6% (71/119)	43.8% (124/283)	0.004
All extrahepatic recurrence	52.1% (62/119)	36.7% (104/283)	0.004

Abbreviations used: CEA, carcinoembryonic antigen; Cryo, cryotherapy; CT, computed tomography; GI, Gastrointestinal; MRI, magnetic resonance imaging; NR, not reported;

Study details

Seifert J K (2005)[3]

Non-randomised controlled trial

Germany

Recruitment period: 1996 to 2002

Study population: Patients with colorectal liver metastases.

n = 223 (55 cryo)

Age: 62 years (mean)

Sex: 58% Male

Patient selection criteria: Cryotherapy used if complete resection of all metastases was not possible, due to: multiple lesions in both lobes, position where resection would sacrifice too much liver parenchyma, or resection too high risk for patient.

Technique: Cryotherapy alone or combined with surgical resection (CMS AccuProbe or CS-6 cryosystem) under ultrasound guidance. Two freeze-thaw cycles used. For larger lesions 2 or more probes were used. In 5 patients laparoscopic approach used. Vs surgical resection only.

Follow-up: 23 months (median)

Conflict of interest/source of funding: not reported

Key efficacy findings

Number of patients analysed: **(55 cryotherapy vs 168 surgical resection)**

Survival

There was no statistically significant difference in median survival between the cryotherapy group (29 months) and the resection group (22 months) (p = 0.56).

There was a statistically significant difference in median disease free survival between the cryotherapy group (6 months) and the resection group (10 months) (p = 0.02).

Outcome	Cryotherapy ± resection n = 55	Surgical resection n =168	p =
Death	49.1% (27/55)	50.6% (85/168)	0.85
Any recurrence	76.4% (42/55)	60.7% (102/168)	0.045
Liver recurrence	70.9% (39/55)	45.2% (76/168)	0.001

Outcome	Cryotherapy only n = 30	Cryotherapy plus surgical resection n = 25	p =
Death	33.3% (10/30)	68.0% (17/25)	0.01
Any recurrence	73.3% (22/30)	80.0% (20/25)	0.56
Liver recurrence	70.0% (21/30)	72.0% (18/25)	0.87

Operative characteristics

Outcome	Cryotherapy n = 55	Resection n = 186	p =
Overall perioperative morbidity	10.9% (6/55)	26.2% (44/168)	0.01
Haemorrhage	n = 2	n = 2	N/R

Key safety findings

Complications

No mortality reported in either group.

Outcome	Cryotherapy n = 55	Resection n = 168	p =
Perioperative mortality	1.8% (1/55)	4.8% (8/168)	0.30
Overall perioperative morbidity	10.9% (6/55)	26.2% (44/168)	0.01
Haemorrhage	n = 2	n = 2	N/R
Wound infection	n = 0	n = 2	N/R
Biloma	n = 1	n = 4	N/R
Abdominal abscess	n = 1	n = 21	N/R
Intestinal fistula	n = 1	n = 3	N/R
Peritonitis	n = 0	n = 1	N/R
Liver failure (transient)	n = 0	n = 6	N/R
GI Haemorrhage	n = 0	n = 3	N/R
Thrombosis	n = 0	n = 2	N/R
Pulmonary embolism	n = 0	n = 2	N/R
Pneumonia	n = 1	n = 1	N/R
Cardiac morbidity (not otherwise described)	n = 0	n = 1	N/R
Acute renal failure	n = 0	n = 1	N/R

Comments

Follow-up issues:

Prospective follow-up.

Loss to follow-up not reported.

Study design issues:

Patients were selected for treatment modality.

Outcomes also evaluated in subgroups of cryotherapy patients who had cryotherapy alone (n = 30) or in combination with resection (n = 25).

Study population issues:

A few patients with resectable lesions who refused resection were treated by laparoscopic cryotherapy.

Significantly more patients in the cryotherapy group had received prior liver resection (p<0.001). Also the lesions in the cryotherapy group were smaller (p < 0.001) and were less frequently synchronous (p = 0.041) due to patient selection.

Other issues:

Adjuvant chemotherapy not routinely given, but in cases of recurrence where repeat local treatment not possible palliative chemotherapy was given.

Abbreviations used: CEA, carcinoembryonic antigen; Cryo, cryotherapy; CT, computed tomography; GI, Gastrointestinal; MRI, magnetic resonance imaging; NR, not reported;

Study details	Key efficacy findings	Key safety findings	Comments
Xu K-C (2008)[4] **Case series** China Recruitment period: 2001 to 2007 Study population: Patients with colorectal liver metastases. Median number of lesions = 1, median size of largest lesion = 3 to 5cm. **n = 326** Age: 55 years (mean) Sex: 75 % Male Patient selection criteria: patients with extrahepatic metastases or liver failure were excluded from the study. Technique: Cryotherapy (with Cryocare system) via percutaneous approach with CT or ultrasound guidance. Two freeze-thaw cycles performed. For larger lesions 2 or more probes were used. Adjuvant transarterial chemo-embolisation in 280 patients. **Follow-up: 32 months (median)** Conflict of interest/source of funding: not reported	Number of patients analysed: **326 cryotherapy** **Survival** Median overall survival was 29 months (range 3 to 62 months). Tumour recurrence occurred in 41.7% (136/326) of patients at a median time to recurrence of 32 months. Overall survival was 78% at 1 year follow up, 41% at 3 years, and 23% at 5 years (absolute figures not reported). Factors that were associated with better survival included tumour < 3cm, right lobe tumour, baseline CEA < 100 ng/dl, and treatment with chemo-embolisation. **Tumour response** 280 patients had CT assessment of tumour response. Complete response was reported in 14.6% (41/280), partial response in 41.1% (115/280), stable disease in 24.3% (68/280), and progressive disease in 20.0% (56/280) (length of follow-up not reported). **Biochemical outcomes** At 30-day follow-up a decrease in CEA level to normal was reported in 77.5% (197/254) of patients. It was increased in 16.1% (41/254) and was unchanged in 6.3% (16/254).	**Complications** Outcome — Rate Mortality — 2.1% (7/326) Hepatic bleeding — 1.5% (5(/326) Cryoshock (mortality) — 0.3% (1/326) Biliary fistula (resolved with drainage) — 0.9% (3/326) Liver failure (mortality) — 0.3% (1/326) Renal insufficiency — 1.5% (5(/326) Liver abscess — 0.9% (3/326) MI and severe arrhythmias — 0.6% (2/326) Pain — 31.6% (103/326) Fever >38°C — 33.1% (108/326) Increased liver enzymes — 38.0% (124/326) Thrombocytopenia — 17.8% (58/326) Pleural effusion — 6.1% (20/326) follow up not reported	**Follow-up issues**: Prospective follow-up protocol. Loss to follow-up not reported. **Study design issues**: Patients with large tumours at baseline, multiple tumours, or high CEA following surgery were given chemo-embolisation. 151 patients underwent repeat cryotherapy for recurrent tumours in the liver or elsewhere. Not all patients underwent all outcome assessments. Not clear if factors associated with survival were analysed by univariate or multivariate analysis. **Study population issues**: None. **Other issues**: 84 patients with liver metastases from other primaries were also treated at the study centre during the recruitment period but are not reported. Authors state that cryotherapy should be regarded as a complement to hepatectomy as an additional means of tumour eradication when total excision cannot be accomplished.

Abbreviations used: CEA, carcinoembryonic antigen; Cryo, cryotherapy; CT, computed tomography; GI, Gastrointestinal; MRI, magnetic resonance imaging; NR, not reported;

Study details	Key efficacy findings	Key safety findings	Comments
Riley D K (1997)[5] **Case series** USA Recruitment period: 1987 to 1995 Study population: Patients with liver metastases from a range of primaries (85% colon). **n = 150 (158 procedures)** Age: 61 years (mean) Sex: 63% Male Patient selection criteria: not reported. Technique: Direct inspection of the liver. With ultrasound guidance cryotherapy probes placed directly into the tumour. One to three freeze-thaw cycles used. Perioperative antibiotics prescribed. **Follow-up: not reported** Conflict of interest/source of funding: not reported	Number of patients analysed: **158 cryotherapy procedures** **Treatment characteristics** Patients who developed infections had a significantly longer length of stay (26 days) than those who did not develop infections (13 days), ($p < 0.001$).	**Complications** Infections occurred following 12.0% (19/158) of procedures. Infections directly related to cryotherapy were reported following 7.6% (12/158) of procedures) (most infections occurred in the first 3 weeks). Outcome — rate Hepatic abscess (intravenous antibiotics required in 5 patients) — 3.8% (6/158) Bile duct obstruction (intravenous antibiotics) — 1.3% (2/158) Intra-abdominal abscess (intravenous antibiotics) — 1.3% (2/158) Cholangitis (intravenous antibiotics) — 0.6% (1/158) Infusaid pump infection (intravenous antibiotics) — 0.6% (1/158) One patient died of multi-organ failure due to malignancy with a secondary infection. There were no infections in 25 patients with biliary tract involvement when the protocol was changed to eliminate cholangiogram or endoscopy as part of routine follow-up.	**Follow-up issues**: Medical records were available for 150 of 156 patients treated. Retrospective study. **Study design issues**: Eight patients underwent repeat cryotherapy treatment. Four surgeons undertook all the procedures. Patient selection for cryotherapy not reported **Study population issues**: One patient who suffered infection was categorised as having primary hepatocellular malignancy rather than liver metastasis. **Other issues**: The data are at least 15 years old, or older, and devices may have evolved during the lapsed period.

Abbreviations used: CEA, carcinoembryonic antigen; Cryo, cryotherapy; CT, computed tomography; GI, Gastrointestinal; MRI, magnetic resonance imaging; NR, not reported;			
Study details	**Key efficacy findings**	**Key safety findings**	**Comments**
Alfredson M J (2005)[6] **Case report** Australia Recruitment period: 2003 Study population: Patient with recurrent colorectal liver metastases **n = 1** Age: 50 years (mean) Sex: 100% Male Patient selection criteria: not reported. Technique: Open cryotherapy. **Follow-up: 2 months** Conflict of interest/source of funding: not reported	Patient with colorectal liver metastasis previously treated with combined resection and cryotherapy. A year later CT imaging revealed a recurrence on top of a previously treated site. Further cryotherapy was undertaken during an open procedure together with removal of a blocked hepatic artery catheter pump. **During the procedure a colonic injury was created while dividing adhesions, which was oversewn.** Postoperative fever abated once a right subphrenic collection was drained. At 3-week follow-up the patient presented at hospital with a short history of rigors but no other symptoms. Liver chemistry demonstrated hepatocellular dysfunction and obstruction, and biliary sepsis was diagnosed. CT scan indicated extensive portal vein gas. Broad spectrum intravenous antibiotics were started. Ten hours later the patient developed septic shock requiring aggressive fluid resuscitation and inotropic support in critical care. The clinical picture improved and 48 hours later CT imaging revealed complete resolution of gas. The patient subsequently developed portal vein thrombosis and MRSA intra-hepatic sepsis. Despite antibiotic treatment and surgical debridement of necrotic liver the patient died from hepatic failure at 2-month follow-up.		**Follow-up issues**: Retrospective study. **Study design issues**: Number of patients treated at the site (denominator) is not reported **Study population issues**: None. **Other issues**: it is not clear whether the colonic injury was the result of cryotherapy application per se, or of the operative approach to the liver, to enable cryotherapy administration'.

Efficacy

An RCT of 123 patients reported that disease-free survival was 14% (9/63) in patients treated with cryotherapy and chemotherapy and 5% (3/60) in patients treated with surgical resection and chemotherapy at 10-year follow-up (measurement of significance not reported)[1]. In the same study overall survival was 19% in the cryotherapy group and 8% in the surgical resection group at 10-year follow-up (absolute figures not reported).

A non-randomised controlled trial of 415 patients reported that there was no statistically significant difference in median survival between the combined resection and cryotherapy group (29 months), and the resection alone group (34 months) ($p = 0.206$)[2]. A non-randomised controlled study of 223 patients reported that there was no statistically significant difference in median survival between patients treated by cryotherapy with or without resection (29 months) and those treated by resection (22 months) ($p = 0.56$)[3]. However median disease-free survival in the cryotherapy with or without resection group (6 months) was significantly shorter than in the resection group (10 months) ($p = 0.02$).

A case series of 326 patients reported that median overall survival was 29 months following cryotherapy for colorectal liver metastases, with tumour recurrence reported in 42% (136/326) of patients at a median time to recurrence of 32 months[4]. In a subset of 280 patients who had computed tomography imaging at baseline and during follow-up, complete response was reported in 14.6% (41/280), partial response in 41.1% (115/280), stable disease in 24.3% (68/280), and progressive disease in 20.0% (56/280) (length of follow-up not reported).

Safety

A non-randomised controlled study of 223 patients reported that there was no statistically significant difference in perioperative mortality between the cryotherapy group (2% [1/55]) and the surgical resection group (5% [8/168]) ($p = 0.30$). However overall perioperative morbidity was significantly lower in the cryotherapy group (11% [6/55] than in the resection group (26% [44/168]) ($p = 0.01$)[3].

A case series of 326 patients reported mortality due to complications related to cryotherapy in 2% (7/326) of patients (follow up period for these events not reported), including 1 patient as a result of ‘cryoshock’ syndrome and 1 patient due to hepatic failure[4].

A case report of 1 patient described extensive portal vein gas following cryotherapy with an open approach, leading to inadvertent colonic perforation and subsequent toxic shock. The patient died from hepatic failure at 2-month follow-up[6].

An RCT of 123 patients reported that pneumonia occurred in 5% (3/63) of patients in the cryotherapy group and in 3% (2/60) of patients in the resection group (measurement of significance not reported)[1]. In a non-randomised controlled study of 223 patients, pneumonia occurred in 1 out of 55 patients in the cryotherapy group and 1 out of 168 patients in the resection group (length of follow up not reported)[3]. In the same study intestinal fistulae developed in 1 of 55 patients and 3 of 168 patients respectively.

An RCT of 123 patients reported severe pain at 5-day follow-up in 0% of patients treated by cryotherapy and 29% of patients treated by resection (absolute figures not reported)[1].

Validity and generalisability of the studies

- Very little RCT data are available.
- Non-randomised controlled studies may suffer from selection bias, making comparison between study groups difficult.
- Few long-term data have been published; local or extrahepatic recurrence may occur at many years' follow-up. However, lack of follow-up may also reflect rapid progression to death in many patients.
- The majority of patients reported in the studies included in table 2 of the overview had liver metastases from colorectal primaries. However there are a small number of patients with liver metastases from other primaries.
- The number of freeze-thaw cycles employed in the treatment protocol varied between studies.
- Many studies report on patients who received additional cryotherapy to treat recurrences during follow-up, making assessment of the efficacy of the index procedure difficult.
- Many patients had surgical resection of liver metastases lesions, in addition to cryotherapy. Also, many patients had systematic or local chemotherapy. Use of adjunct or sequential treatments makes inferences about efficacy and safety more difficult.

- Some studies are relatively old – surgical techniques (for liver excision, either as an adjunct procedure, or as a comparator) have evolved, and the same can be true for cryotherapy devices and/or technique.
- No evidence was identified comparing cryotherapy directly (head-to-head) with other thermal ablation techniques.

Existing assessments of this procedure

A Cochrane systematic review of 'resection versus no intervention or other surgical interventions for colorectal cancer liver metastases' by Al-asfoor A (2008)[7] includes only 1 RCT comparing resection and cryotherapy (Karpan 1997), which is included in table 2 below.

Related NICE guidance

Below is a list of NICE guidance related to this procedure. Appendix B gives details of the recommendations made in each piece of guidance listed.

Interventional procedures

- Radiofrequency ablation for the treatment of colorectal liver metastases. NICE interventional procedures guidance 327 (2009). Available from www.nice.org.uk/IPG327
- Microwave ablation for the treatment of metastases in the liver. NICE interventional procedures guidance 220 (2007). Available from www.nice.org.uk/IPG220
- Selective internal radiation therapy for colorectal metastases in the liver. NICE interventional procedures guidance 93 (2004). Available from www.nice.org.uk/IPG093. This guidance is currently under review and is expected to be updated in 2010. For more information, see www.nice.org.uk/IPG093

Specialist Advisers' opinions

Specialist advice was sought from consultants who have been nominated or ratified by their Specialist Society or Royal College. The advice received is their individual opinion and does not represent the view of the society.

Mr D Lloyd (Association of Upper Gastrointestinal Surgeons of GB and Ireland)
Mr G Poston (Association of Upper Gastrointestinal Surgeons of GB and Ireland).

- One Specialist Adviser commented that the procedure is outdated and has been completely abandoned in the UK, and the other commented that it is a

minor variation on an established procedure, which is unlikely to alter that procedure's safety and efficacy.

- The main comparators for this procedure are radiofrequency and microwave ablative techniques.
- There have been reports of major haemorrhage with this procedure, which sometimes led to patient death, the rare but fatal complication of cryoshock syndrome, and a high local recurrence rate. It therefore fell out of use, particularly with the development of radiofrequency and lately microwave ablation.
- Anecdotal adverse events following this procedure include cryoshock that may lead to multi-organ failure, liver fracture, damage to bile ducts, damage to structures outside the liver, and bleeding.
- The key efficacy outcome for this procedure would be tumour destruction with small margin of additional destruction, and survival.
- Surgeons and radiologists should be trained appropriately for this procedure.

Patient Commentators' opinions

NICE's Patient and Public Involvement Programme sent questionnaires to 2 trusts for distribution to patients who had the procedure (or their carers). NICE received no completed questionnaires.

Issues for consideration by IPAC

- Cryotherapy has been delivered during open resection, percutaneously, and laparoscopically.
- The extent to which cryotherapy is used as a stand-alone therapy or as an add-on to conventional resection is not clear. It is rarely used as part of a hepatic resection procedure these days as radiofrequency and microwave ablation have become more popular.

- It is only used as ‘stand-alone’ treatment by the few centres that currently have access to the percutaneous probes.

References

1 Korpan NN. (1997) Hepatic cryosurgery for liver metastases. Long-term follow-up. Annals of Surgery 225:193–201.

2 Niu R, Yan TD, Zhu JC et al. (2007) Recurrence and survival outcomes after hepatic resection with or without cryotherapy for liver metastases from colorectal carcinoma. Annals of Surgical Oncology 14:2078–2087.

3 Seifert JK, Springer A, Baier P et al. (2005) Liver resection or cryotherapy for colorectal liver metastases: a prospective case control study. International Journal of Colorectal Disease 20:507–520.

4 Xu K-C, Niu L-Z, He W-B et al. (2008) Percutaneous cryosurgery for the treatment of hepatic colorectal metastases. World Journal of Gastroenterology 14:1430–1436.

5 Riley DK, Babinchak TJ, Zemel R et al. (1997) Infectious complications of hepatic cryosurgery. Clinical Infectious Diseases 24:1001–1003.

6 Alfredson MJ, Brooks AJ, Talbot ML et al. (2005) Hepatic portal vein gas as a complication of cryotherapy. Journal of the International Hepato-Pancreato-Biliary Association 7:159–160.

7 Al-Asfoor A, Fedorowicz Z, and Lodge M. (2008) Resection versus no intervention or other surgical interventions for colorectal cancer liver metastases. Cochrane Database of Systematic Reviews CD006039-

Appendix A: Additional papers on cryotherapy for the treatment of liver metastases

The following table outlines the studies that are considered potentially relevant to the overview but were not included in the main data extraction table (table 2). It is by no means an exhaustive list of potentially relevant studies.

Article	Number of patients/follow-up	Direction of conclusions	Reasons for non-inclusion in table 2
Awad N, Ross WB, Preketes AP et al. (1994) Effect of peri-operative blood transfusion on survival after hepatic cryotherapy (for metastatic colorectal cancer). Surgical Research Communications 16 (1) 59–63	Non-randomised controlled study n = 47 Follow-up = 420 days	Perioperative blood transfusion does not influence survival after hepatic cryotherapy for metastatic colorectal cancer.	Larger studies are included in table 2
Bageacu S, Kaczmarek D, Lacroix M et al. (2007) Cryosurgery for resectable and unresectable hepatic metastases from colorectal cancer. European Journal of Surgical Oncology 33 (5) 590–596	Case series n = 53 Follow-up = 2 years	Survival rates were comparables between patients with resectable and unresectable metastases, but a high complication rate and a substantial rate of local recurrence following cryosurgery should caution against its use to treat resectable disease.	Larger studies are included in table 2
Brooks AJ, Wang F, Alfredson M. (2005) Synchronous liver resection and cryotherapy for colorectal metastases: survival analysis. Surgeon Journal of the Royal Colleges of Surgeons of Edinburgh & Ireland 3 (4) 265–268	Case series n = 86 Follow-up = 18 months	Patients with liver metastases that are not amenable to resection alone can achieve worthwhile median survival with synchronous liver resection and cryotherapy ablation.	Larger studies are included in table 2
Charnley RM, Doran J, and Morris DL.(1989) Cryotherapy for liver metastases: a new approach. British Journal of Surgery 76 (10) 1040–1041	Case series n = 7 Follow-up = 1 to 6 months	Cryotherapy produced an area of destruction within the liver substance which decreased in size by 6 weeks as shown on serial computed tomographic scanning.	Larger studies are included in table 2
Chen YY, Perera DS, Yan TD, et al. (2006) Applying Fong's CRS liver score in patients with colorectal liver metastases treated by cryotherapy. Asian Journal of Surgery 29 (4) 238–241	Case series n = 61 Follow-up = 3 years	The clinical risk score (CRS) score can be used to predict outcome of hepatic cryotherapy, but the difference in survival between CRS 2, 3 and 4 is modest.	Larger studies are included in table 2

Cozzi PJ, Englund R, and Morris DL. (1995) Cryotherapy treatment of patients with hepatic metastases from neuroendocrine tumors. Cancer 76 (3) 501–509	Case series n = 6 Follow-up = 24 months	To the authors' knowledge, this study demonstrates for the first time that hepatic cryotherapy offers supportive treatment for patients with neuroendocrine tumours metastatic to the liver. Cryotherapy alleviates symptoms and may improve survival.	Larger studies are included in table 2
Dale PS, Souza JW, and Brewer DA. (1998) Cryosurgical ablation of unresectable hepatic metastases. Journal of Surgical Oncology 68 (4) 242–245	Case series n = 6 Follow-up = 17 months	Cryosurgical ablation is a safe method of treating unresectable hepatic malignancies and it may extend survival in carefully selected patients.	Larger studies are included in table 2
Dwerryhouse SJ, Seifert JK, McCall JL et al. (1998) Hepatic resection with cryotherapy to involved or inadequate resection margin (edge freeze) for metastases from colorectal cancer. British Journal of Surgery 85 (2) 185–187	Case series n = 26 Follow-up = 23 months	Cryotherapy to involved or inadequate resection margins improves local disease control considerably.	Larger studies are included in table 2 Possibly the same patients as Niu (2007)
Finlay IG, Seifert JK, Stewart GJ et al. (2000) Resection with cryotherapy of colorectal hepatic metastases has the same survival as hepatic resection alone. (Erratum appears in Eur J Surg Oncol Aug;26(5): 524–5). European Journal of Surgical Oncology 26 (3) 199–202	Non randomised controlled study n = 107 (75 cryo) Follow-up = not reported	Edge and contra-lobe cryotherapy can be combined with hepatic resection to allow a greater proportion of patients with hepatic colorectal metastases to be offered treatment, and results in similar survival figures comparable to hepatic resection for at least 3 years.	Larger studies are included in table 2
Goering JD, Mahvi DM, Niederhuber JE et al. (2002) Cryoablation and liver resection for noncolorectal liver metastases. American Journal of Surgery 183 (4) 384–389	Case series n = 42 Follow-up = 5 years	Cryosurgical hepatic tumour ablation for metastatic non-colorectal primary tumours results in survival and local hepatic tumour recurrence rates similar to resection alone.	Larger studies are included in table 2
Huang A, McCall JM, Weston MD, et al. (2002) Phase I study of percutaneous cryotherapy for colorectal liver metastasis. British Journal of Surgery 89 (3) 303–310	Non randomised controlled study n = 22 Follow-up = not reported	Percutaneous cryotherapy for treatment of colorectal liver metastases is feasible and may have a place in conjunction with chemotherapy.	Larger studies are included in table 2
Johnson LB, Krebs , Wong-You-Cheong J et al. (1997) Cryosurgical debulking of unresectable liver metastases for palliation of carcinoid syndrome. Surgery 121 (4) 468–470	Case report n = 1 Follow-up = 6 months	At 6 months the patient continued to have amelioration of carcinoid syndrome.	Larger studies are included in table 2

Johnson LB, Krebs TL, van Echo D, et al. (1997) Cytoablative therapy with combined resection and cryosurgery for limited bilobar hepatic colorectal metastases. American Journal of Surgery 174 (6) 610–613	Non randomised controlled study n = 14 Follow-up = 8 months	Cytoablation of hepatic metastases can be safely achieved with combined hepatic resection and cryosurgery in selected patients. Long-term survival data are necessary before advocating widespread application of this approach.	Larger studies are included in table 2
Joosten J, Jager G, Oyen W, et al. (2005) Cryosurgery and radiofrequency ablation for unresectable colorectal liver metastases. European Journal of Surgical Oncology 31 (10) 1152–1159	Non randomised controlled study n = 58 (30 cryo) Follow-up = 26 months	More widespread use of these techniques seems promising but requires further investigation in randomized trials comparing local ablative treatment with chemotherapy.	Larger studies are included in table 2
Kornprat P, Jarnagin WR, DeMatteo RP et al. (2007) Role of intraoperative thermoablation combined with resection in the treatment of hepatic metastasis from colorectal cancer. Archives of Surgery 142 (11) 1087–1092	Non randomised controlled trial n = 39 (20 cryo) Follow-up = 21 months	In these patients with extensive bilobar disease, recurrence rates are high, but long-term survival is encouraging and may be improved with aggressive postoperative chemotherapy.	Larger studies are included in table 2
Mala T, Edwin B, Mathisen O, et al. (2004) Cryoablation of colorectal liver metastases: minimally invasive tumour control. Scandinavian Journal of Gastroenterology 39 (6) 571-578	Case series n = 19 Follow-up = 10 months	Short-term tumour control can be achieved following cryoablation of colorectal liver metastases. A minimally invasive approach is feasible but the diameter of metastases considered for percutaneous cryoablation should not exceed 3 cm.	Larger studies are included in table 2
Morris DL and Ross WB. (1996) Australian experience of cryoablation of liver tumors: metastases. Surgical Oncology Clinics of North America 5 (2) 391–397	Case series n = 149 Follow-up = not reported	There was only one 30-day death; morbidity was modest. Postoperative carcinoembryonic antigen (CEA) changes were extremely predictive of outcome in patients with liver metastases from colorectal cancer.	Larger studies are included in table 2 Possibly the same patients as Niu (2007)
Onik GM, Atkinson D, Zemel R et al. (1993) Cryosurgery of liver cancer. Seminars in Surgical Oncology 9 (4) 309–317	Case series n = 57 Follow-up = 6 months minimum	Although the results are still short-term, this study indicates that hepatic cryosurgery offers the hope of long-term survival in patients with unresectable hepatic metastases.	Larger studies are included in table 2
Osada S, Imai H, Yawata K. (2008) Growth inhibition of unresectable tumors induced by hepatic cryoablation: Report of two cases. Hepato-Gastroenterology 55 (81) 231–234	Case report n = 2 Follow-up = 5 to 21 months	The efficacy of percutaneous cryosurgery (PCS) as a treatment strategy for unresected liver tumour was evaluated in two cases.	Larger studies are included in table 2

Pearson AS, Izzo F, Fleming R. YD et al. (1999) Intraoperative radiofrequency ablation or cryoablation for hepatic malignancies. American Journal of Surgery 178 (6) 592–599	Non-randomised controlled study n = 146 (54 cryo) Follow-up = 15 months	This study indicates that complications occur much less frequently following radiofrequency ablation of liver tumours compared with cryoablation of liver tumours, and early local tumour recurrence is infrequent.	Larger studies are included in table 2
Ravikumar TS, Steele G,Jr, Kane R et al, (1991) Experimental and clinical observations on hepatic cryosurgery for colorectal metastases. Cancer Research 51 (23: Pt 1)	Case series n = 24 Follow-up = 2 years	These data demonstrate that cryosurgery is a useful modality for treating unresectable primary and metastatic liver cancers.	Larger studies are included in table 2
Ravikumar TS, Kane R, Cady B. (1987) Hepatic cryosurgery with intraoperative ultrasound monitoring for metastatic colon carcinoma. Archives of Surgery 122 (4) 403–409	Case series n = 10 Follow-up = 8 months	This study establishes the technical feasibility and antitumour response of hepatic cryosurgery.	Larger studies are included in table 2
Redlich PN, Baker EJ, McAuliffe TL. (2006) Surgical management of colorectal metastases to the liver: role of resection and cryosurgery. Wisconsin Medical Journal 95 (12) 859–863	Non-randomised controlled study n = 62 (21 cryo) Follow-up = 44 months	By multivariate analysis, only transfusions correlated significantly with survival, but in a negative manner ($p = 0.05$).	Larger studies are included in table 2
Rivoire M, De Cian F, Meeus, P et al. (2000) Cryosurgery as a means to improve surgical treatment of patients with multiple unresectable liver metastases. Anticancer Research 20 (5C) 3785-3790	Case series n = 19 Follow-up = 28 months	Cryosurgery may be effective in the treatment of patients with multiple unresectable liver metastases and should be investigated in multimodality treatment programmes.	Larger studies are included in table 2
Ruers TJ, Joosten J, Jager GJ, et al. (2001) Long-term results of treating hepatic colorectal metastases with cryosurgery. British Journal of Surgery 88 (6) 844–849	Case series n = 30 Follow-up = 26 months	In patients with colorectal liver metastases, local ablative techniques can be used as an effective adjunct to hepatic resection to obtain tumour clearance.	Larger studies are included in table 2
Schlinkert RT and Chapman TP.(1990) Nitrogen embolus as a complication of hepatic cryosurgery. Archives of Surgery 125 (9) 1214	Case report n = 1 Follow-up = to discharge	Modifications of liquid nitrogen probes, which prevent direct contact of liquid nitrogen with the tissue being frozen, can prevent this type of injury.	Larger studies are included in table 2

Seifert JK, Cozzi PJ, and Morris DL (1998) Cryotherapy for neuroendocrine liver metastases. Seminars in Surgical Oncology 14 (2) 175–183	Case series n = 13 Follow-up = 14 months	This study shows that hepatic cryotherapy offers a useful treatment option for this group of patients, alleviates symptoms and may have an impact on survival.	Larger studies are included in table 2
Seifert JK and Morris DL. (1998) Cryotherapy of the resection edge after liver resection for colorectal cancer metastases. Australian & New Zealand Journal of Surgery 68 (10) 725-728	Case series n = 44 Follow-up = 19 months	Cryotherapy of the resection edge after resection of colorectal liver metastases with involved or inadequate resection margins considerably improves local disease control.	Larger studies are included in table 2
Seifert JK and Morris DL. (1998) Prognostic factors after cryotherapy for hepatic metastases from colorectal cancer. Annals of Surgery 228 (2) 201-208	Case series n = 116 Follow-up = 20 months	Hepatic cryotherapy is a safe and effective treatment option for patients with non-resectable liver metastases from colorectal cancer, with promising results regarding survival.	Larger studies are included in table 2
Seifert JK, Achenbach T, Heintz A, et al (2000) Cryotherapy for liver metastases. International Journal of Colorectal Disease 15 (3) 161-166	Case series n = 49 Follow-up = not reported	Hepatic cryotherapy is associated with tolerable morbidity and mortality. Efficacy is demonstrated by tumour marker results. Survival data are promising; however, long-term results must be provided to allow comparison with other treatment modalities.	Larger studies are included in table 2
Stella M, Mithieux F, Meeus P et al. (2006) Transpleurodiaphragmatic cryosurgical ablation for recurrent unresectable colorectal liver metastases. Journal of Histotechnology 28 (4) 268–272	Case series n = 13 Follow-up = 26 months	Transpleurodiaphragmatic cryotherapy is safe and effective in selected patients with unresectable recurrent liver metastases from colorectal cancer.	Larger studies are included in table 2
Wallace JR, Christians KK, Pitt HA et al. (1999) Cryotherapy extends the indications for treatment of colorectal liver metastases. Surgery 126 (4) 766–774	Non randomised controlled study n = 106 (72 cryo) Follow-up = 14 months	This study demonstrates improved long-term survival for 'cured' patients with more than 4 metastatic lesions, thereby extending the indications for resection/ablation.	Larger studies are included in table 2
Wallis CB and Coventry DM. (1997) Anaesthetic experience with laparoscopic cryotherapy. A new technique for treating liver metastases. Surgical Endoscopy 11 (10) 979–981	Case series n = 5 Follow-up = to discharge	Laparoscopic hepatic cryotherapy can be performed without significant temperature changes, but it entails significant morbidity.	Larger studies are included in table 2

Weaver ML, Atkinson D, and Zemel R. (1995) Hepatic cryosurgery in the treatment of unresectable metastases. Surgical Oncology 4 (5) 231-236	Case series n = 140 Follow-up = 27 months	The median survival for all patients was 22 months. Of those patients followed for more than 2 years, the median survival was 25 months.	Larger studies are included in table 2
Wong LL, Limm WM, Cheung AH. (1995) Hepatic cryosurgery: early experience in Hawaii. Hawaii Medical Journal 54 (12) 811–813	Case report n = 4 Follow-up = 6 to 11 months	All patients currently are alive with up to 11-month follow-up. Long-term studies will be necessary to assess the effectiveness of this modality.	Larger studies are included in table 2
Yan TD, Nunn DR, and Morris DL. (2006) Recurrence after complete cryoablation of colorectal liver metastases: analysis of prognostic features. American Surgeon 72 (5) 382-390	Case series n = 135 Follow-up = 30 months	Resection plus cryoablation rather than cryoablation alone should be used for larger lesions.	Larger studies are included in table 2
Yeh KA, Fortunato L, Hoffman JP et al. (1997) Cryosurgical ablation of hepatic metastases from colorectal carcinomas. American Surgeon 63 (1) 63-68	Case series n = 24 Follow-up = 19 months	Cryoablation of unresectable hepatic metastases or close resection margins is safe and may allow for improved survival in selected patients with metastatic colon and rectal carcinoma.	Larger studies are included in table 2
Zhou XD, Tang ZY, Yu YQ et al. (1993) The role of cryosurgery in the treatment of hepatic cancer: a report of 113 cases. Journal of Cancer Research & Clinical Oncology 120 (1-2) 100–102	Case series n = 6 Follow-up = not reported	These results indicate that cryosurgery, the in situ freezing of cancer, is a safe and effective treatment for unresectable hepatic cancer.	Larger studies are included in table 2

Appendix B: Related NICE guidance for cryotherapy for the treatment of liver metastases

Guidance	Recommendations
Interventional procedures	**Radiofrequency ablation for the treatment of colorectal metastases in the liver. NICE interventional procedures guidance 327 (2009)** 1.1 Current evidence on the safety and efficacy of radiofrequency (RF) ablation for colorectal liver metastases is adequate to support the use of this procedure in patients unfit or otherwise unsuitable for hepatic resection, or in those who have previously had hepatic resection, provided that normal arrangements are in place for clinical governance, consent and audit. 1.2 Patient selection should be carried out by a hepatobiliary cancer multidisciplinary team **Microwave ablation for the treatment of metastases in the liver. NICE interventional procedures guidance 220 (2007)** 1.1 Current evidence on the safety and efficacy of microwave ablation for the treatment of metastases in the liver does not appear adequate for this procedure to be used without special arrangements for consent and for audit or research. 1.2 Clinicians wishing to use microwave ablation for the treatment of metastases in the liver should take the following actions. • Inform the clinical governance leads in their Trusts. • Ensure that patients understand the uncertainty about the procedure's safety and efficacy and provide them with clear written information, including about other treatment options. In addition, use of the Institute's information for patients ('Understanding NICE guidance') is recommended. • Audit and review clinical outcomes of all patients having microwave ablation for the treatment of metastases in the liver (see section 3.1). 1.3 Patient selection should be carried out by a multidisciplinary team that includes a hepatobiliary surgeon.

	1.4 The procedure should be performed under appropriate imaging guidance. 1.5 As a number of devices are available, and there is some uncertainty about the energy levels that should be used, any adverse events relating to this procedure should be reported to the Medicines and Healthcare products Regulatory Agency. 1.6 Further research on the procedure would be useful. The Institute may review the procedure upon publication of further evidence. **Selective internal radiation therapy for colorectal metastases in the liver. NICE interventional procedures guidance 093 (2004)** 1.1 Current evidence on the safety of selective internal radiation therapy (SIRT) for colorectal metastases in the liver appears adequate. With regard to efficacy, the procedure may reduce tumour bulk, but there is a lack of evidence of symptom relief or increased survival, and combination with other treatments makes interpretation of the published literature difficult. 1.2 Clinicians wishing to undertake selective internal radiation therapy for colorectal metastases in the liver should take the following actions. • Ensure that patients understand the uncertainty about the procedure's safety and efficacy and provide them with clear, written information. Use of the Institute's *Information for the Public* is recommended. • Audit and review clinical outcomes of all patients having selective internal radiation therapy for colorectal metastases in the liver. 1.3 Publication of research studies with outcome measures which include survival will be useful in reducing the current uncertainty about the efficacy of the procedure. The Institute may review the procedure upon publication of further evidence.

Appendix C: Literature search for cryotherapy for the treatment of liver metastases

Database	Date searched	Version/files
Cochrane Database of Systematic Reviews – CDSR (Cochrane Library)	26/01/10	January 2010
Database of Abstracts of Reviews of Effects – DARE (CRD website)	26/01/10	N/A
HTA database (CRD website)	26/01/10	N/A
Cochrane Central Database of Controlled Trials – CENTRAL (Cochrane Library)	26/01/10	January 2010
MEDLINE (Ovid)	26/01/10	1950 to January Week 2 2010
MEDLINE In-Process (Ovid)	26/01/10	January 25, 2010
EMBASE (Ovid)	26/01/10	1980 to 2010 Week 03
CINAHL (NHS Evidence)	26/01/10	1981 to Present
BLIC (Dialog DataStar)	22/01/10	1995 to date

The following search strategy was used to identify papers in MEDLINE. A similar strategy was used to identify papers in other databases.

1	Cryotherapy/
2	Cryosurgery/
3	(Cryotherap* or Crymotherap* or Cryoblat* or Cryosurg* or Cryotherm* or Cryotreatment* or Cryoprocedure* or Cryoprob* or Cryoneedle* or Cryocare*).tw.
4	((Cold* or Cryogenic*) adj3 (Therap* or Procedure*)).tw.
5	((Tumour* or Tumor*) adj3 Ablation*).tw.
6	((Freez* or Thaw*) adj3 (Techni* or Surg* or Procedure* or Cycle*)).tw.
7	((Ice adj3 Ball*) or Ice-Ball* or Ice Ball*).tw.

8	Seednet*.tw.
9	or/1-7
10	Liver Neoplasms/
11	Neoplasm Metastasis/
12	10 and 11
13	(Liver* adj3 (Neoplasm* or Cancer* or Metastas*)).tw.
14	or/12-13
15	9 and 14
16	Carcinoma, Hepatocellular/
17	Hepatocellular*.tw.
18	16 and 17
19	15 not 18
20	Animals/ not Humans/
21	19 not 20