A multidisciplinary approach to improve treatment strategies for patients with hepatic or pancreatic cancer

Leede, E.M. de

CHAPTER 3

Perfusion with high dose chemotherapy in patients with unresectable liver metastases of uveal melanoma: results from two experienced centers

E.M. de Leede, M.C. Burgmans, H.W. Kapiteijn, M.J. Jager,
A.R. van Erkel, F.G.J. Tyl, D. Grünhagen, J. Rothbarth, C. Verhoef,
C.J.H. van de Velde and A.L. Vahrmeijer
Melanoma Research, December 2016

ABSTRACT

Objective

Uveal melanoma patients have a poor survival after diagnosis of metastatic disease. Isolated hepatic perfusion (IHP) was developed to treat patients with unresectable metastases confined to the liver. This retrospective analysis focusses on treatment characteristics, complications, toxicity and survival after IHP.

Methods

Patients with uveal melanoma metastases confined to the liver treated with IHP in two experienced hepato-pancreatic-biliary surgery centers (EMC and LUMC) were included.

Results

Between March 1999 and April 2009, 30 patients were treated with IHP. The duration of surgery was 3.7 hours (EMC) versus 8.7 hours (LUMC) and also the dosage of melphalan differed; 1 mg/kg body weight (n=12) versus a dose of 170-200 mg (n= 18) or melphalan (100 mg) combined with oxaliplatin (50 or 100 mg) (n=3). The length of hospital stay was 10 days. Two patients developed occlusion of the hepatic artery, and died respectively 3 days and 1.5 month after surgery. Progression free survival was 6 (1-16) months and recurrences occurred mainly in the liver. Median overall survival was 10 (3-50) months.

Conclusions

IHP is a potentially beneficial treatment modality resulting in a reasonable overall survival for uveal melanoma patients. Because of substantial morbidity related to the open procedure, a percutaneous system has been developed and is currently being investigated.

INTRODUCTION

Uveal melanoma arises from melanocytes in the ocular chorioid, ciliary body or iris of the eye. It is the most common primary intraocular malignant tumour in adults and the age at diagnosis is most often between 55 and 65 years. [1] Intraocular tumours are detected incidentally or present with visual symptoms and are diagnosed using fundoscopic and ultrasound examination by an ophthalmologist. The treatment of the primary tumour consists of local radiotherapy (brachytherapy, proton beam irradiation or stereotactic radiotherapy) or enucleation of the eye. After treatment of the primary tumour with no synchronous metastases, patients are kept under surveillance often with half-yearly liver function tests and hepatic ultrasound. Up to 62% of the patients may develop metastases, most commonly or solely in the liver [2, 3, 4]Liver metastases are the life-limiting risk factor for these patients. [5] The median survival after diagnosis of metastatic disease in the liver is poor: 2-12 months without treatment and 10-12 months after loco-regional chemotherapy-based treatments. [4, 5, 6, 7, 8, 9] The survival time after detection of metastatic disease is significantly associated with several factors such as tumour burden, symptoms of the metastases, length of interval between treatment of primary tumour and detection of metastases, liver function and patients performance score. [8 10]

Currently, surgical resection of liver metastases is the gold standard for any patient with 'liver only' disease. However, most uveal melanoma patients do not meet the criteria for resection because the metastases are spread diffusely throughout the liver or because of excessive (miliary) tumour burden. Besides surgery, treatment options are limited and currently there is no standard treatment available for patient with uveal melanoma metastases. Systemic therapy, such as dacarbazine (DTIC), is used to treat patients with metastatic disease, but results have been disappointing. [11] Singh *et al.* reported that the 5-year relative survival in the United States did not improve over time from 1973-2008 despite the development of new agents. [12] New treatment options like targeted therapy and immunotherapy are widely investigated in clinical trials, but effectiveness in uveal melanoma is as yet unclear. [13] Besides systemic therapy and surgery, locoregional treatments are being investigated, such as radiofrequency ablation (RFA), microwave ablation, isolated hepatic perfusion (IHP), selective internal radiation therapy with Yttrium-90 microspheres and trans-arterial chemoembolization (TACE). These locoregional modalities could be implemented in the treatment plan of patients with uveal melanoma metastases, since the metastases are often confined to the liver. Furthermore, the rare complete responses that have been reported, were achieved with local therapies, indicating the value of these modalities.[8]

IHP was developed about thirty years ago to treat patients with unresectable metastases from various origin confined to the liver.[14] The principle of IHP is to isolate the liver from the systemic circulation and perfuse it with high dose chemotherapy. Systemically administered this high dose chemotherapy could potentially cause fatal complications.[15] The advantage of IHP as a whole liver treatment is the fact that all (micro) metastases are being treated whereas other local treatment modalities often only target detectable tumours. Many patients with unresectable uveal melanoma and especially colorectal cancer liver metastases have been treated with IHP with radiological response rates ranging from 50 to 62%. [16, 17, 18, 19, 20, 21]

Two University Medical Centers in the Netherlands have an IHP program since the early nineties and gained experience with this procedure; IHP has been performed during laparotomy in over 130 patients with liver metastases (colorectal cancer, uveal melanoma, neuroendocrine tumours, GIST, HCC etc.)[16, 17, 21, 22]. The aim of the study was to investigate the efficacy of this treatment for patients with uveal melanoma metastases confined to the liver. In this paper, we describe the results of treating 30 patients with uveal melanoma liver metastases with IHP in two centers using melphalan (in some cases combined with oxaliplatin) as chemotherapeutic agent.

METHODS

Patient selection criteria

All patients with uveal melanoma metastases who were treated with IHP in either the Erasmus MC Cancer Institute (EMC) or the Leiden University Medical Center (LUMC) were selected for this retrospective analysis. Treatment of the primary tumour (enucleation or radiotherapy) had been performed prior to entering the study protocol. The liver metastases had to be unresectable and were considered so on the basis of multiple lesions (>10) in multiple segments and/or a location near vascular structures, making an oncological resection impossible, as seen on imaging (CT or MRI). Moreover, all patients were discussed in a multidisciplinary meeting (radiologist, medical oncologist, surgeon, pathologist).

Tumour involvement had to be less than 50% of liver tissue, determined by volumetric measurements by the radiologist, to prevent massive necrosis and subsequent organ failure in case of a good response. All patients had to be above 18 years of age and have a World Health Organization (WHO) performance status of 0 or 1, liver enzymes (ALAT, ASAT and alkaline phosphatase) less than five times the upper limit of normal (ULN) and bilirubin not higher than twice the ULN. In case a patient did not meet one of the criteria, he or she was not included in the

trial. Exclusion criteria were age over 70 years, evidence of extrahepatic disease on CT scan of thorax and abdomen, and administration of chemotherapy within four weeks prior to the IHP treatment. Routinely, angiography was performed prior to IHP to exclude aberrant hepatic arteries or to visualize other anatomic anomalies, as well as to screen for secondary signs of portal hypertension, such as hepatofugal flow. The study protocol was approved by the Medical Ethical Committee of both centers and informed consent was obtained from all patients.

Chemotherapeutic agents

A dosage of 1 mg/kg melphalan was used in the EMC, based on a study by Verhoef. [22] Doses of 170-200 mg were given to the LUMC patients (Alkeran, Wellcome Pharmaceuticals B.V., Utrecht, The Netherlands), based on an earlier phase I study of IHP with melphalan, where a total dose of 200 mg appeared to be the maximally tolerated dose. [23] Also, in the LUMC patients have been treated in a dose-escalation trial; 50 or 100 mg of oxaliplatin (Sanofi-Aventis, Gouda, The Netherlands) was added to a fixed dose of 100 mg melphalan. In all cases melphalan was infused into the perfusion circuit through a side-line. In case of patients treated in the dose escalation study, the oxaliplatin was administered as a bolus before melphalan infusion.

Surgical procedure

The patients were treated with a single IHP procedure as described previously: in the EMC as described by Verhoef *et al*. in 2008 [22] *(Figure 1)* and in the LUMC as described by Rothbarth *et al.* in 2003 [16] and Vahrmeijer *et al*. in 2000 [23] *(Figure 2)*. After laparotomy, the portal vein (PV) and proper hepatic artery (HA) were dissected and the HA artery was cannulated via the gastroduodenal artery followed by heparinization of the blood. The inferior caval vein (ICV) was isolated and clamped above the renal veins and below the diaphragm to prevent venous leakage. Tourniquets/clamps were also secured around the HA and PV to isolate the hepatic circuit. The HA and PV catheters were connected to the perfusion circuit.

Melphalan was infused into the perfusion circuit using an infusion pump and IHP was performed under mild hyperthermic conditions (39°C). After one hour period of perfusion a wash-out procedure was performed. Finally, all cannulas and clamps were removed and normal circulation was restored and all incisions were closed. In the EMC the portal vein was cannulated for outflow; resulting in a hypoxic technique, with retrograde outflow, hence isolated hypoxic hepatic perfusion (IHHP). An aortic clamp was placed for controlling systemic blood pressure. A constant flow perfusion (of approximately 350 ml/min, mean) under pressure monitoring was established.

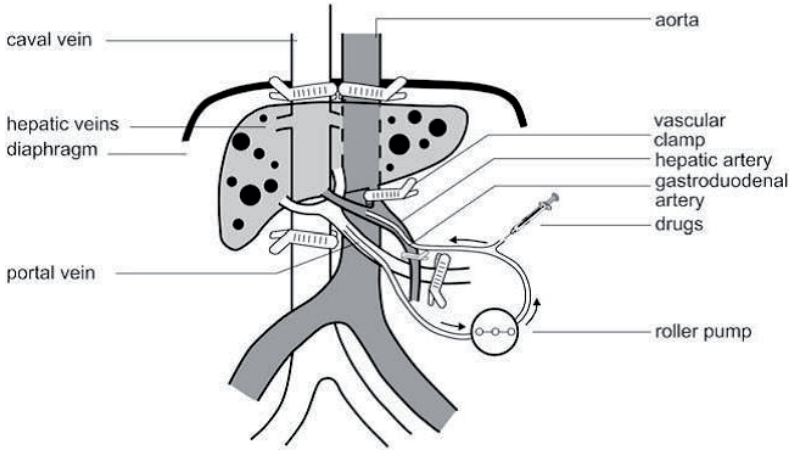

FIGURE 1. **The retrograde perfusion setup, as used in the Erasmus University Medical Center**

During the procedure at the LUMC the perfusate was oxygenated using a heart–lung machine. An extracorporeal veno-venous bypass was used to maintain circulation in the abdomen and the lower extremities. To achieve this, the right femoral vein and the PV were cannulated proximal to a tourniquet and connected to the right axillary vein. To prevent possible post-operative cholecystitis, a cholecystectomy was performed routinely.

Leakage detection

Leakage of perfusate into the systemic circuit was monitored using a radioactive tracer (10 MBq 99mTc-pertechnetate, 99mTc). This was injected into the isolated circuit with subsequent measurement of the radioactivity levels in both the systemic and isolated circuit as previously described [24, 25]. Systemic leakage was continuously monitored with a scintillation counter and was expressed quantitatively as a percentage. If no leakage was detected, the chemotherapeutic agent(s) were administered. Leakage during perfusion was allowed to be 10%. If this level was reached, perfusion was immediately stopped.

Postoperative care and follow-up

Patients were monitored in the intensive care unit for at least one day after IHP. Liver and kidney function tests, such as ALAT, ASAT, bilirubin, lactate dehydrogenase (LDH), alkaline phosphatase, creatinine, urea, number of platelets and white blood cell count were measured frequently. Toxicity was graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events Criteria (CTCAE

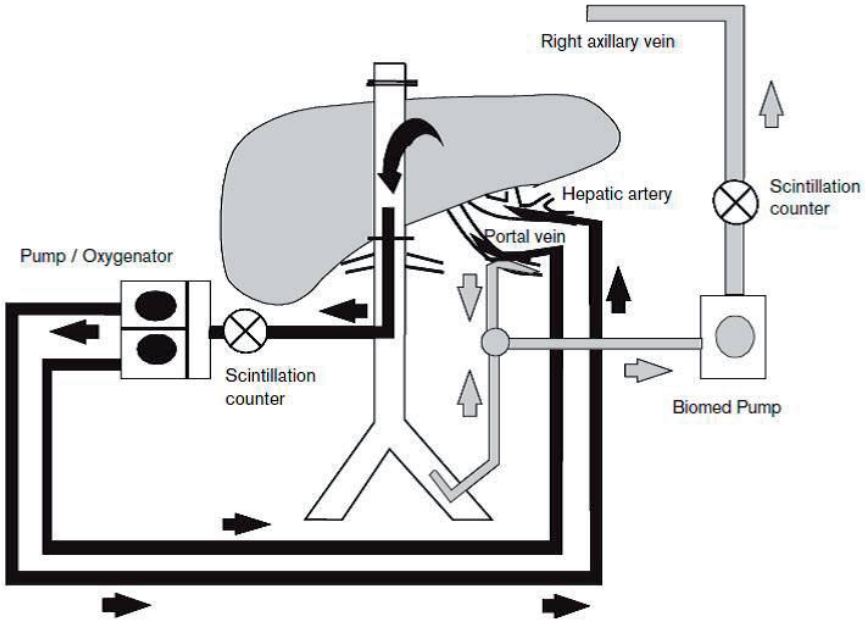

FIGURE 2. **Isolated hepatic perfusion with extracorporeal veno-venous bypass, as used in the Leiden University Medical Center.**

v4.0). Granulocyte colony-stimulating factor (G-CSF, Filgrastim/Neupogen®, Amgen B.V., Breda, The Netherlands) was administered, however not routinely.

Response evaluation

Tumour response was evaluated by comparing post-procedural abdominal contrast-enhanced CT and/or MRI scans at three month intervals with pre-perfusion scans. Progressive disease was defined an increase in size of ≥25% or the appearance of new intra- or extrahepatic lesions.

Statistical Analysis

All data were analysed using SPSS software for Windows version 20 (SPSS, Chicago, Illinois, USA).

RESULTS

Patient characteristics *(Table 1)*

Between March 1999 and April 2009, 31 patients with histologically proven uveal melanoma with metastases confined to the liver underwent surgery for isolated hepatic perfusion in either the EMC or LUMC. Biopsies of the liver lesion(s) were

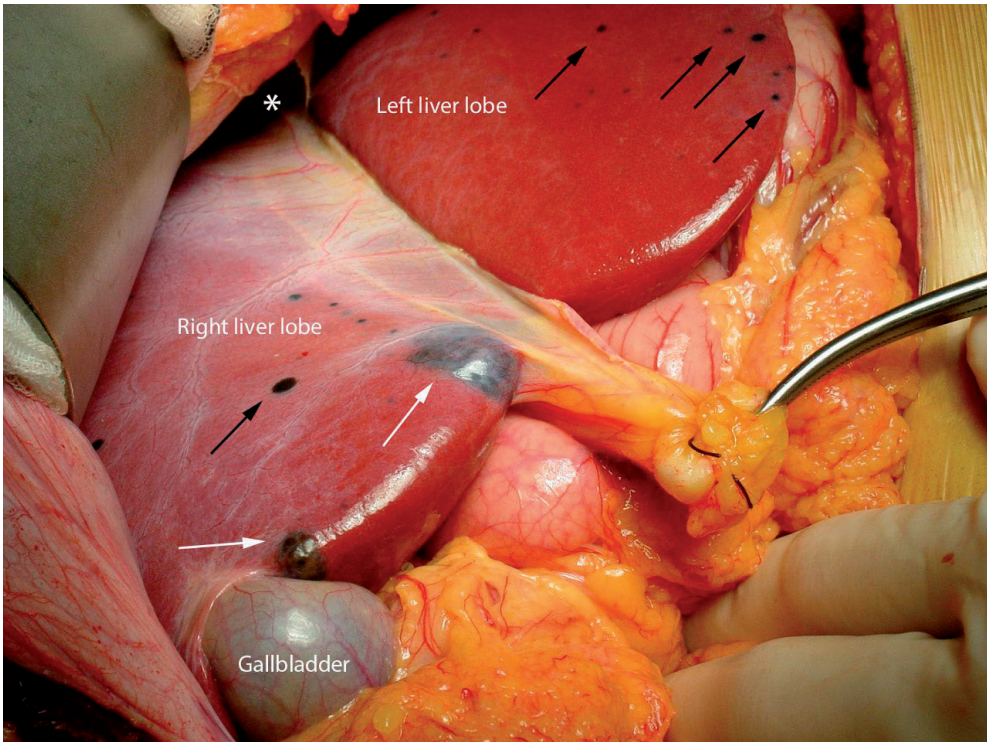

FIGURE 3. **Per-operative photograph of the liver. Black spots (pointed by arrows) are uveal melanoma metastases; black arrows indicate small lesions, most likely not seen on CT-scan, white arrows indicate larger metastases. Picture was taken in a cranial direction from the right side of the patient (asterix in direction of head of the patient).**

obtained to proof that the suspected hepatic lesions seen on imaging or during surgery were indeed melanoma metastases. The median age at the time of treatment was 57 years. Treatment of the primary tumour was mostly enucleation and most patients developed liver metastases metachronously. Most patients had multiple metastases (over 10) and/or metastases diffusely spread throughout the liver (see Figure 3). Four patients received previous treatment of the liver metastases: one patient received dacarbazine and three patients trial-related immunotherapy (consisting of GM-CSF, IL-2 and IFNalpha). The time interval between the primary diagnosis of uveal melanoma and the clinical diagnosis of liver metastases ranged from synchronous liver metastases at time of diagnosis of the primary tumour up to eleven years (range 0-133 months). Metastases were detected during routine 3 or 6 monthly follow-up visits including liver enzyme blood tests and ultrasound of the abdomen.

TABLE I. **Patient characteristics and history.**

No. of patients included	**31**
Male : female	**12:19**
Median age at time of primary diagnosis , years, range) Median age at time of treatment, years, range)	**53 (27-68)** **57 (28-70)**
Treatment of primary tumour (number of patients) Enucleation Local stereotactic irradiation Local Ruthenium plaque Proton beam treatment	 **18** **4** **8** **1**
Liver metastases (number of patients) Metachronous Synchronous	 **29** **2**
Median time from primary diagnosis to metastases, months (range)	**27 (0-133)**
Previous treatment of liver metastases (number of patients) Dacarbazine° Immunotherapy/trial	 **1** **3**
Median time from diagnosis of liver metastases to IHP, weeks (range)	**10 (4-58)**

(°DTIC – dacarbazine, an alkylating oncolyticum)

Surgical characteristics *(Table 2)*

One procedure in the LUMC was aborted before melphalan infusion because of systemic leakage of the radioactive tracer; this patient did not receive chemotherapy and was therefore excluded from further survival analysis. The dosage of melphalan differed between the two centers. The 12 patients in the EMC received a dose of 1mg/kg body weight (dose 60 – 95 mg). In the LUMC 15 patients were treated with 170-200 mg of melphalan, and 3 patients in a combined melphalan–oxaliplatin dose-escalation study: 2 patients with a combination of 100 mg melphalan and 50 mg of oxaliplatin, and 1 patient with 100 mg melphalan and 100 mg of oxaliplatin. [26] Median time of surgery was 3.7 hours (2.6 – 4.8) in the EMC and 8.4 hours (6.2 – 10.2) in the LUMC. At the EMC, the IHHP procedure was performed without a veno-venous bypass and a heart-lung machine. Consequently an extracorporeal perfusionist is not needed and operation time and blood loss are reduced using this method.

Complications and toxicity

One patient died three days postoperatively because of liver failure caused by occlusion of the hepatic artery leading to multi-organ failure. One patient was discharged with impaired liver functions because of an occluded hepatic artery and died 1.5 months after surgery. Veno-occlusive disease (VOD) occurred in two patients who both had a 7 months survival after surgery. One patient developed

TABLE 2. **Treatment characteristics and survival (n=30 patients)**

	Total (n=30)	**EMC (n=12)**	**LUMC (n= 18[‡])**
Dose chemotherapeutic agent : melphalan (mg) (O.: Oxaliplatin)		65-95 mg	170-200 mg (n=13) 100 mg & O. 50 mg (n=2) 100 mg & O. 100 mg (n=1)
Leakage (median)		n.r.	0.5%
Operation time (median, hours)		3.7 hours	8.4 hours
Perioperative blood loss (median, ml)		700[*] ml	3500 ml
Hospital stay (median)	**10 days**		
Postoperative treatment (no. of patients) Systemic therapy Ablation	**8** **2**		
Progression-free survival (median, (range))	**6 months (1-16)**		
Localisation of progression (no. of patients) Hepatic Extrahepatic Both hepatic and extrahepatic	**14** **4** **10**		
Overall survival (median, months (range)) IHP until death Diagnosis of liver metastasis until death Diagnosis of primary tumour until death	**10 months (0-50)** **13.5 months (2-53)** **39 months (11-149)**		

(n.r.=not reported of most patients, [‡] One procedure aborted in LUMC; no further analysis, [*]missing of four patients)

non-infectious fever postoperatively that resolved within a few days. Hepatic toxicity consisted of a transient rise of liver enzymes. Systemic toxicity was mainly leukopenia, with CTCAE grade 0-1 and grade 4 in one patient.

Progression-free and overall survival

Results of progression-free survival and overall survival of the 30 treated patients are shown in Table 2. Median progression-free survival after IHP was 6 months (range 1-16) and progression was hepatic (14/30), both hepatic and extrahepatic (10/30) or extrahepatic (4/30). Extra-hepatic progression consisted of lung and bone metastasis and skin metastasis in 14 patients. Median overall survival after IHP treatment was 10 months (range 0-50 months). Median overall survival from diagnosis of liver metastasis was 13.5 (range 2-53) months. Besides the two patients that died postoperatively of liver failure, all patients died because of progression of metastatic disease. The 1-year survival for this cohort was 41.9% and the 2-year

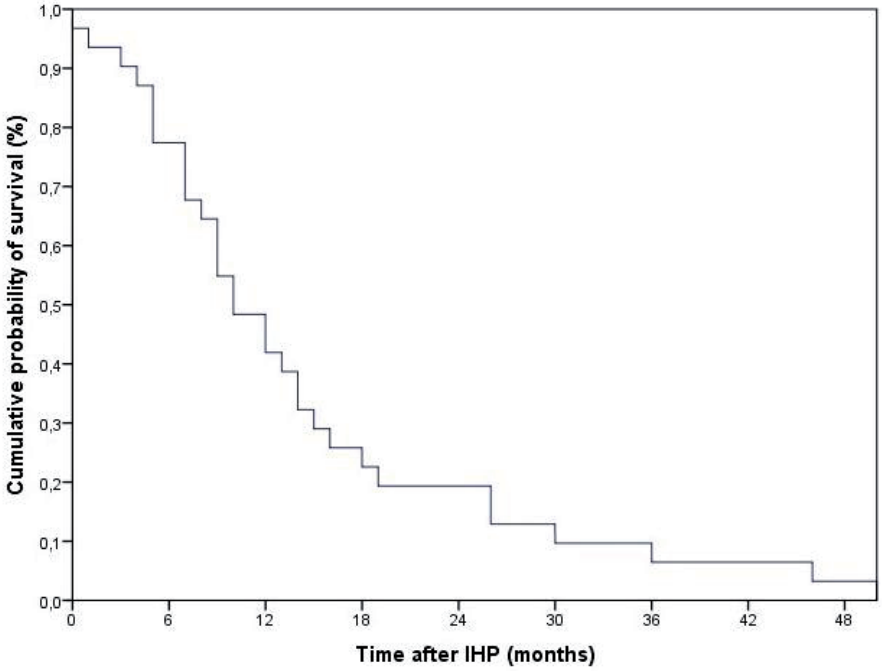

FIGURE 4. **Kaplan-Meier curve for overall survival after isolated hepatic perfusion with melphalan. All patients combined. (n=*31*) *One patient died 1.5 days after the procedure and therefor at time point 0.**

survival was 19.4% as shown in the Kaplan-Meier curve in figure 4. Median overall survival from primary tumour diagnosis was 39 months (range 11-149). Ten patients received postoperative (systemic) treatment after diagnosis of disease progression. The other patients did not receive systemic therapy often at their own wish or due to rapidly progressive disease. Since no standard treatment modality was (and still is) available, the only option for treatment was to participate in phase I/II trial protocols.

DISCUSSION

This study presents the results of 30 patients with unresectable liver metastases of uveal melanoma treated with isolated hepatic perfusion with melphalan in two experienced centers. For this selected group of patients, the median overall survival was 13.5 months after diagnosis of liver metastases and the 1-year survival was 41.9%.

Augsburger *et al. (2009)* listed 20 prospective studies with several treatment modalities (chemotherapy; systemic and applied locally to the liver, and

chemoembolization) for patients with metastatic uveal melanoma. The study groups were of comparable size to our study and the median overall survival was 5.0- 24 months (for prospective studies). Our study with 30 patients treated with I(H)HP fits in the middle of these listed studies with a median overall survival of 13.5 months. The median survival for unselected case series was even worse; 3.6-15 months. However, this might be a better representation of reality, since the prospective studies describe the results in a study population. [6] Previously reported median overall survival after diagnosis of metastatic disease in the liver was 4.2-12.5 months if untreated, with a 1-year survival of 13-20%. [8, 10, 27, 28, 29] For patients that received treatment, mostly in phase I/II study protocols, the median overall survival increased to 5.2-27 months. Most of these studies contain selected patient groups. [28, 29]

Compared to several other treatment modalities, intrahepatic treatment was associated with prolonged survival. [30 31] Current literature reports on new treatment modalities, such as dendritic cell vaccination and new (application of excisting) chemotherapy, however the results of research on these new modalities have not been confirmed in large trials yet. [32, 33] Based on the above mentioned data we conclude that patients might benefit from I(H)HP compared to untreated patients and possibly have a longer overall survival compared to other treatment modalities. These data should be judged with caution as case selection could have influenced the results: the median age at the time of diagnosis (53 years) of the primary tumour in our series was lower than in most reported series of uveal melanoma. The average age of uveal melanoma patients reported in previous studies is 61 years old, and in high risk cases 59. [1 34] Also, the group consisted mostly of women, although uveal melanoma does not show a sex preponderance.

In order for isolated perfusion of the liver to become an acknowledged treatment option for liver only metastases, peri-operative morbidity needs to be reduced, most likely by adapting the procedure. Firstly, the I(H)HP procedure performed during laparotomy, is associated with morbidity due to the 'open' approach and the invasive manner of clamping and cannulating various blood vessels. In the current analysis, four patients experienced a thromboembolic adverse event or veno-occlusive disease and two patients died from the consequences of hepatic artery occlusion. By creating a different approach, the laparotomy-associated morbidity could be prevented. A second adjustment to the procedure should concern 'repeatability', because the predominant site of progression or recurrence is the liver. Indeed, a possible way to achieve longer progression free survival is repeating the IHP treatment. In case of an 'open' IHP procedure, adhesions and effects on the vascular anatomy of the cannulation and clamping impede repetition. Already in 1994 Ravikumar *et al.* investigated a percutaneous in-human approach for isolated liver

perfusion. [35] Several studies report on a less invasive, percutaneous approach, but had disappointing results, for instance because the occlusion balloon methodology failed to obtain leakage control.[36] Due to lack of evidence of efficacy, the technique was largely abandoned until the early 2000's when it was re-evaluated by the National Cancer Institute (NCI) in the United States.[37] A renewed method of isolated hepatic perfusion was developed recently, which isolates the liver from the systemic circulation using a new system of percutaneous placed catheters. Percutaneous hepatic perfusion (PHP) with chemosaturation is minimally invasive, has limited systemic toxicity combined with high local drug exposure like IHP and has been performed up to 8 times. [38, 39] This new approach meets the two improvements needed as mentioned above: (1) change to a minimally invasive procedure and (2) repeatability. Recent studies using PHP for uveal melanoma report a 50% complete and partial response rate, and improved hepatic progression free survival (7 versus 1.6 months) after percutaneous hepatic perfusion compared to best alternative care. [40 41] Our centers are currently investigating this new technique in a two-center phase II trial aiming to treat uveal melanoma patients with unresectable liver metastases

CONCLUSION

We have analysed the results of isolated (hypoxic) hepatic perfusion in treating 30 patients with unresectable liver metastases of uveal melanoma treated from 1999-2009. Patients treated with IHP seem to benefit from IHP compared to no treatment and equally compared to other treatment modalities. Because of substantial morbidity related to the open procedure, a percutaneous method has been developed and is currently being investigated.

REFERENCES

1. Shields, C.L., S. Kaliki, M. Furuta, E. Fulco, C. Alarcon, and J.A. Shields, *American Joint Committee on Cancer Classification of Uveal Melanoma (Anatomic Stage) Predicts Prognosis in 7731 Patients: The 2013 Zimmerman Lecture.* Ophthalmology, 2015. 122(6): p. 1180-1186.
2. Jovanovic, P., M. Mihajlovic, J. Djordjevic-Jocic, S. Vlajkovic, S. Cekic, and V. Stefanovic, *Ocular melanoma: an overview of the current status.* Int J Clin Exp Pathol, 2013. 6(7): p. 1230-1244.
3. Woodman, S.E., *Metastatic uveal melanoma: biology and emerging treatments.* Cancer J, 2012. 18(2): p. 148-152.
4. Collaborative Ocular Melanoma Study, G., *Assessment of metastatic disease status at death in 435 patients with large choroidal melanoma in the Collaborative Ocular Melanoma Study (COMS): COMS report no. 15.* Arch Ophthalmol, 2001. 119(5): p. 670-676.
5. Gragoudas, E.S., K.M. Egan, J.M. Seddon, R.J. Glynn, S.M. Walsh, S.M. Finn, et al., *Survival of patients with metastases from uveal melanoma.* Ophthalmology, 1991. 98(3): p. 383-389; discussion 390.
6. Augsburger, J.J., Z.M. Correa, and A.H. Shaikh, *Effectiveness of treatments for metastatic uveal melanoma.* Am J Ophthalmol, 2009. 148(1): p. 119-127.
7. Olofsson, R., L. Ny, M.S. Eilard, M. Rizell, C. Cahlin, U. Stierner, et al., *Isolated hepatic perfusion as a treatment for uveal melanoma liver metastases (the SCANDIUM trial): study protocol for a randomized controlled trial.* Trials, 2014. 15: p. 317.
8. Rietschel, P., K.S. Panageas, C. Hanlon, A. Patel, D.H. Abramson, and P.B. Chapman, *Variates of survival in metastatic uveal melanoma.* J Clin Oncol, 2005. 23(31): p. 8076-8080.
9. Vahrmeijer, A.L., C.J. van de Velde, H.H. Hartgrink, and R.A. Tollenaar, *Treatment of melanoma metastases confined to the liver and future perspectives.* Dig Surg, 2008. 25(6): p. 467-472.
10. Diener-West, M., S.M. Reynolds, D.J. Agugliaro, R. Caldwell, K. Cumming, J.D. Earle, et al., *Screening for metastasis from choroidal melanoma: the Collaborative Ocular Melanoma Study Group Report 23.* J Clin Oncol, 2004. 22(12): p. 2438-2444.
11. Pons, F., M. Plana, J.M. Caminal, J. Pera, I. Fernandes, J. Perez, et al., *Metastatic uveal melanoma: is there a role for conventional chemotherapy?–A single center study based on 58 patients.* Melanoma Res, 2011. 21(3): p. 217-222.
12. Singh, A.D., M.E. Turell, and A.K. Topham, *Uveal melanoma: trends in incidence, treatment, and survival.* Ophthalmology, 2011. 118(9): p. 1881-1885.
13. Moser, J.C., J.S. Pulido, R.S. Dronca, R.R. McWilliams, S.N. Markovic, and A.S. Mansfield, *The Mayo Clinic experience with the use of kinase inhibitors, ipilimumab, bevacizumab, and local therapies in the treatment of metastatic uveal melanoma.* Melanoma Res, 2014.
14. van de Velde, C.J., B.J. Kothuis, H.W. Barenbrug, N. Jongejan, R.D. Runia, L.M. de Brauw, et al., *A successful technique of in vivo isolated liver perfusion in pigs.* J Surg Res, 1986. 41(6): p. 593-599.

15. Noter, S.L., J. Rothbarth, M.E. Pijl, J.E. Keunen, H.H. Hartgrink, F.G. Tijl, et al., *Isolated hepatic perfusion with high-dose melphalan for the treatment of uveal melanoma metastases confined to the liver.* Melanoma Res, 2004. 14(1): p. 67-72.
16. Rothbarth, J., M.E. Pijl, A.L. Vahrmeijer, H.H. Hartgrink, F.G. Tijl, P.J. Kuppen, et al., *Isolated hepatic perfusion with high-dose melphalan for the treatment of colorectal metastasis confined to the liver.* Br J Surg, 2003. 90(11): p. 1391-1397.
17. van Iersel, L.B., H. Gelderblom, A.L. Vahrmeijer, E.L. van Persijn van Meerten, F.G. Tijl, H. Putter, et al., *Isolated hepatic melphalan perfusion of colorectal liver metastases: outcome and prognostic factors in 154 patients.* Ann Oncol, 2008. 19(6): p. 1127-1134.
18. Alexander, H.R., Jr. and C.C. Butler, *Development of isolated hepatic perfusion via the operative and percutaneous techniques for patients with isolated and unresectable liver metastases.* Cancer J, 2010. 16(2): p. 132-141.
19. Alexander, H.R., S.K. Libutti, D.L. Bartlett, M. Puhlmann, D.L. Fraker, and L.C. Bachenheimer, *A phase I-II study of isolated hepatic perfusion using melphalan with or without tumor necrosis factor for patients with ocular melanoma metastatic to liver.* Clin Cancer Res, 2000. 6(8): p. 3062-3070.
20. van Iersel, L.B., E.J. Hoekman, H. Gelderblom, A.L. Vahrmeijer, E.L. van Persijn van Meerten, F.G. Tijl, et al., *Isolated hepatic perfusion with 200 mg melphalan for advanced noncolorectal liver metastases.* Ann Surg Oncol, 2008. 15(7): p. 1891-1898.
21. van Etten, B., J.H. de Wilt, F. Brunstein, A.M. Eggermont, and C. Verhoef, *Isolated hypoxic hepatic perfusion with melphalan in patients with irresectable ocular melanoma metastases.* Eur J Surg Oncol, 2009. 35(5): p. 539-545.
22. Verhoef, C., J.H. de Wilt, F. Brunstein, A.W. Marinelli, B. van Etten, M. Vermaas, et al., *Isolated hypoxic hepatic perfusion with retrograde outflow in patients with irresectable liver metastases; a new simplified technique in isolated hepatic perfusion.* Ann Surg Oncol, 2008. 15(5): p. 1367-1374.
23. Vahrmeijer, A.L., J.H. van Dierendonck, H.J. Keizer, J.H. Beijnen, R.A. Tollenaar, M.E. Pijl, et al., *Increased local cytostatic drug exposure by isolated hepatic perfusion: a phase I clinical and pharmacologic evaluation of treatment with high dose melphalan in patients with colorectal cancer confined to the liver.* Br J Cancer, 2000. 82(9): p. 1539-1546.
24. Runia, R.D., L.M. de Brauw, B.J. Kothuis, E.K. Pauwels, and C.J. van de Velde, *Continuous measurement of leakage during isolated liver perfusion with a radiotracer.* Int J Rad Appl Instrum B, 1987. 14(2): p. 113-118.
25. Marinelli, A., L.M. de Brauw, H. Beerman, H.J. Keizer, J.H. van Bockel, U.R. Tjaden, et al., *Isolated liver perfusion with mitomycin C in the treatment of colorectal cancer metastases confined to the liver.* Jpn J Clin Oncol, 1996. 26(5): p. 341-350.
26. van Iersel, L.B., E.M. de Leede, A.L. Vahrmeijer, F.G. Tijl, J. den Hartigh, P.J. Kuppen, et al., *Isolated hepatic perfusion with oxaliplatin combined with 100 mg melphalan in patients with metastases confined to the liver: A phase I study.* Eur J Surg Oncol, 2014. 40(11): p. 1557-1563.
27. Diener-West, M., S.M. Reynolds, D.J. Agugliaro, R. Caldwell, K. Cumming, J.D. Earle, et al., *Development of metastatic disease after enrollment in the COMS trials for treatment of choroidal melanoma: Collaborative Ocular Melanoma Study Group Report No. 26.* Arch Ophthalmol, 2005. 123(12): p. 1639-1643.

28. Augsburger, J.J., Z.M. Correa, and A.H. Shaikh, *Quality of evidence about effectiveness of treatments for metastatic uveal melanoma.* Trans Am Ophthalmol Soc, 2008. 106: p. 128-135; discussion 135-127.
29. Eskelin, S., S. Pyrhonen, M. Hahka-Kemppinen, S. Tuomaala, and T. Kivela, *A prognostic model and staging for metastatic uveal melanoma.* Cancer, 2003. 97(2): p. 465-475.
30. Buzzacco, D.M., M.H. Abdel-Rahman, S. Park, F. Davidorf, T. Olencki, and C.M. Cebulla, *Long-term survivors with metastatic uveal melanoma.* Open Ophthalmol J, 2012. 6: p. 49-53.
31. Olofsson, R., C. Cahlin, C. All-Ericsson, F. Hashimi, J. Mattsson, M. Rizell, et al., *Isolated hepatic perfusion for ocular melanoma metastasis: registry data suggests a survival benefit.* Ann Surg Oncol, 2014. 21(2): p. 466-472.
32. Bol, K.F., H.W. Mensink, E.H. Aarntzen, G. Schreibelt, J.E. Keunen, P.G. Coulie, et al., *Long overall survival after dendritic cell vaccination in metastatic uveal melanoma patients.* Am J Ophthalmol, 2014. 158(5): p. 939-947.
33. Carvajal, R.D., G.K. Schwartz, H. Mann, I. Smith, and P.D. Nathan, *Study design and rationale for a randomised, placebo-controlled, double-blind study to assess the efficacy of selumetinib (AZD6244; ARRY-142886) in combination with dacarbazine in patients with metastatic uveal melanoma (SUMIT).* BMC Cancer, 2015. 15: p. 467.
34. Piperno-Neumann, S., V. Servois, P. Mariani, C. Plancher, C. Levy-Gabriel, L. Lumbroso-Le Rouic, et al., *Prospective study of surveillance testing for metastasis in 100 high-risk uveal melanoma patients.* J Fr Ophtalmol, 2015. 38(6): p. 526-534.
35. Ravikumar, T.S., G. Pizzorno, W. Bodden, J. Marsh, R. Strair, J. Pollack, et al., *Percutaneous hepatic vein isolation and high-dose hepatic arterial infusion chemotherapy for unresectable liver tumors.* J Clin Oncol, 1994. 12(12): p. 2723-2736.
36. van Etten, B., F. Brunstein, I.M.G. van, A.W. Marinelli, C. Verhoef, J.R. van der Sijp, et al., *Isolated hypoxic hepatic perfusion with orthograde or retrograde flow in patients with irresectable liver metastases using percutaneous balloon catheter techniques: a phase I and II study.* Ann Surg Oncol, 2004. 11(6): p. 598-605.
37. Lillemoe, H.A. and H.R. Alexander, Jr., *Current Status of Percutaneous Hepatic Perfusion as Regional Treatment for Patients with Unresectable Hepatic Metastases: A Review.* Am Oncology and Hematology Rev 2014. 2014(15-23).
38. Miao, N., J.F. Pingpank, H.R. Alexander, S.M. Steinberg, T. Beresneva, and Z.M. Quezado, *Percutaneous hepatic perfusion in patients with metastatic liver cancer: anesthetic, hemodynamic, and metabolic considerations.* Ann Surg Oncol, 2008. 15(3): p. 815-823.
39. Jones, A. and H.R. Alexander, Jr., *Development of isolated hepatic perfusion for patients who have unresectable hepatic malignancies.* Surg Oncol Clin N Am, 2008. 17(4): p. 857-876, x.
40. Vogl, T.J., S. Zangos, J.E. Scholtz, F. Schmitt, S. Paetzold, J. Trojan, et al., *Chemosaturation with percutaneous hepatic perfusions of melphalan for hepatic metastases: experience from two European centers.* Rofo, 2014. 186(10): p. 937-944.
41. Hughes, M.S., J. Zager, M. Faries, H.R. Alexander, R.E. Royal, B. Wood, et al., *Results of a Randomized Controlled Multicenter Phase III Trial of Percutaneous Hepatic Perfusion Compared with Best Available Care for Patients with Melanoma Liver Metastases.* Ann Surg Oncol, 2015.


3