Local ablative therapies for colorectal liver metastases and the immune system

Duijnhoven, Frederieke van

Chapter 1

General Introduction

Partly adapted from F.H. van Duijnhoven, R.I.J.M. Aalbers, P.J.K. Kuppen and O.T. Terpstra. The immunological consequences of photodynamic therapy; a literature review. Immunobiology 2003;207(2):105-113

Locoregional treatment of colorectal liver metastases

Colorectal cancer is one of the most common malignancies in the western world and is associated with a poor prognosis. This is mainly due to the occurrence of metastases, predominantly in the liver. Synchronous liver metastases are found in 10 to 25% of patients[1] and eventually over 70% of patients will develop liver metastases[2,3]. Without treatment, these patients have a poor life expectancy of 5 to 9 months[4-7].
If metastases are confined to the liver, surgical removal of the liver metastases is a possible curative treatment option. Unfortunately only 20 to 30% of patients with liver metastases are eligible for resection, as number, size and location of liver metastases often preclude resection[8]. However, several other locoregional treatment modalities such as local ablative therapies and locoregional chemotherapy may offer palliation, prolongation of survival or even curation in this patient group.

Hepatic resection
Currently, resection of liver metastases is the gold standard treatment for colorectal liver metastases. Only patients with liver metastases that can be resected with tumour-free margins and with sufficient functioning liver volume are eligible for resection, which drastically limits the applicability of this curative treatment. However, the restriction of inadequate remaining liver volume may be overcome by selective chemoembolization of the portal vein branches feeding the liver lobe in which the tumour is located[9]. Upon embolization, hypertrophy of the remaining liver lobe is induced, increasing the functioning liver volume to an acceptable percentage.

Resection of liver metastases is associated with morbidity rates of 8-24%, mainly related to the major abdominal operation it implies[10-15]. Complications related specifically to liver resection include bile leak, bile fistulas and formation of perihepatic abscess. Postoperative mortality rate is less than 4%, with the main causes of death being peroperative haemorrhage, infection and liver failure. Results of hepatic resection have been extensively reported, with large studies showing 5-year survival rates of 27–37 %[12,16-19]. However, up to 20% of patients develop new liver tumours or extrahepatic disease after hepatic resection[20-22]. Prognosis after resection is strongly associated with obtaining tumour-free resection margins, as the extent of the resection margin correlates with a better prognosis[23-27]. Other factors that influence prognosis are the stage of the primary tumour, disease-free interval, number and size of liver metastases, preoperative CEA and extrahepatic disease[28-33].

Local ablative techniques

Various local ablative techniques are available for the treatment of colorectal liver metastases. Radiofrequency ablation (RFA) and cryotherapy are currently most often applied, other modalities include laser induced thermotherapy (LITT), photodynamic therapy (PDT), percutaneous alcohol injection (PAI) and stereotactic radiotherapy.

Radiofrequency ablation – In RFA, needle electrodes deliver a high frequency alternating current to the tissue, causing hyperthermia of the tissue and thus inducing coagulative necrosis. RFA electrodes can be either single probes, inducing a cylindrical necrotic lesion, or multi-tined expandable electrodes that induce spherical lesions. Similar to resection, an adequate necrotic margin surrounding the tumour should be achieved by RFA. This often requires multiple insertions when tumours are over 3 cm in diameter and emphasizes the pivotal importance of correct placement of the electrodes. Electrodes are usually placed under ultrasound guidance, either percutaneously or in an open procedure. The latter may be preferred when correct placement of electrodes is hindered (large tumours, compromised electrode accessibility) or when there is an increased risk of complications (tumours close to large vessels, diaphragm or to adjacent internal organs). Additionally, RFA can be easily applied in combination with surgical resection when there is bilobar distribution of liver tumours that cannot all be surgically removed. Thus, RFA broadens the applicability of resection, enabling potentially curative treatment in more liver metastases patients than the present 20-30%.

RFA also has considerable merits on its own, as it has resulted in complete response rates of 52-95%. It can offer palliation by prolongation of disease-free and overall survival to respectively 50% and 94% at 1 year, with a median survival time of 30-34 months[34-40]. Possibly it can result in curation, although at present the limited follow-up time in most studies does not allow a meaningful determination of survival rates. Until there is further research available into the comparison of RFA with conventional surgery, resection is still considered to be the gold standard therapy for colorectal liver metastases. However, RFA can be applied when resection is not possible due to intimate association with major blood vessels, or when the medical condition of the patient or previous hepatic resection hampers a large open procedure.

Cryoablation – For cryoablation, a probe through which liquid nitrogen circulates is inserted in the lesion, repeatedly freezing and thawing the surrounding tumour tissue. The resulting formation of intra- and extracellular ice crystals causes tumour destruction. Median survival of around 26 months has been reported[41-45] but morbidity rate is considerable, ranging from 10 to 30%, with bleeding of the liver as most serious complication. Several studies have compared RFA with cryotherapy, and confirmed the high rates of local recurrence and complications after cryosurgery[46,47]. In addition, RFA probes are of smaller diameters

than those used in cryotherapy, which facilitates the applicability of RFA. Most clinicians therefore seem to prefer RFA to cryotherapy and the use of RFA now greatly exceeds that of cryotherapy.

Laser induced thermotherapy & photodynamic therapy – In smaller and more experimental settings, LITT and PDT have also shown to be able to adequately treat liver tumours. LITT resembles the RFA technique, as it is based on the generation of heat in the tumour. However, heat is not generated by high frequency current but by a laser applicator that delivers light energy through optical fibres inserted in the target tissue. The resulting coagulative necrosis leads to tumour destruction[48]. Studies have shown tumour responses up to 97% after 6 months, with median survival ranging from 32 to 39 months[49-52].

Similarly to LITT, PDT makes use of optical fibres and laser light. Contrary to LITT and RFA, antitumour effect is not based on generation of heat but of reactive oxygen species. In PDT, a photosensitising agent is administered systemically and will, with varying specificity, localize in tumour tissue[53,54]. Upon subsequent tumour illumination by light of an appropriate wavelength, the photosensitiser is excited by photons to an unstable higher energy level. When returning to its ground state energy level, the absorbed energy is transferred to oxygen, which leads to the formation of reactive oxygen species. These reactive oxygen species are cytotoxic and cause direct tumour cell damage and vascular damage, resulting in both apoptosis and necrosis[55]. PDT has been successfully applied in a rat model for liver metastases[56,57] and results of a phase I trial show PDT to be safe, feasible and effective in treatment of colorectal liver metastases as well[58].

Percutaneous alcohol injection – Another technique involving percutaneous insertion of probes directly into the tumour is the intratumoural injection of alcohol. PAI is widely used in the treatment of HCC with tumour response rates up to 80%[59], but its role in treatment of colorectal liver metastases is not as well defined. For PAI, sterile ethyl alcohol is injected though a needle that is placed in the tumour under ultrasound-guidance. The alcohol will cause chemical coagulative necrosis, followed by formation of fibrotic tissue and thrombosis of small intratumoural vessels. In liver metastases from various primary tumours, complete necrosis is obtained in 52-58% of treated tumours[60,61] and median survival is 26 months. The more solid aspect of colorectal liver metastases however impairs the adequate injection of sufficient volumes of alcohol in the tumours. This is corroborated by the poor results of PAI in these tumours, with no necrosis induced in 22 tumours[62]. Although complications are minimal, PAI does not seem to be a very promising technique in treatment of colorectal liver metastases, due to its low antitumour efficacy.

Stereotactic radiotherapy – Stereotactic radiotherapy is a technique which value is not yet assessed in large clinical trials. As the liver has a low tolerance dose for radiation and studies concerning whole-liver irradiation for liver metastases did not show any survival benefit, radiotherapy was until recently not a part of standard nor experimental treatment for colorectal liver metastases. However, improvements in patient

positioning and 3D planning software have enabled stereotactic treatment of liver tumours, with a single high dose of radiotherapy being delivered to a specific focus in the liver with minimal damage to healthy liver tissue[48,63-66].

Results of a phase I/II trial with single-dose stereotactic radiotherapy for liver tumours were reported by Herfarth et al[67]. A total of 60 liver tumours, half of which were colorectal liver metastases, were treated with this technique without complications and with local control at 6 weeks achieved in 98% of the tumours. Although at present there is no substantial evidence indicating that results with radiotherapy are comparable to those achieved with other local ablative therapies, further clinical studies may advocate stereotactic radiotherapy as a new possible treatment modality in colorectal liver metastases.

Locoregional chemotherapy

If resection or local ablative therapy of colorectal liver metastases is not possible, patients may benefit from systemic chemotherapy. Systemic toxicity of chemotherapeutics however limits the use of these antitumour drugs. Administration of cytostatic drugs directly into the hepatic artery may overcome these problems, as systemic drug exposure is limited. Hepatic tumours derive their blood supply mainly from the hepatic artery and not from the portal system, as opposed to liver parenchyma. This allows for high doses to be delivered in the tumour while exposure of liver parenchyma is limited[68,69]. Chemotherapeutics may be infused into the hepatic artery with or without further isolation of the liver circulation. In hepatic artery administration (HAI), drugs are administered into the hepatic artery without isolation of the liver circulation. Most commonly, fluorodeoxyuridine (FUDR) or 5-fluorouracil (5-FU) are used, with tumour response rates of up to 74%[70-74]. However, HAI does not increase overall or progression-free survival when compared to systemic chemotherapy and its clinical use seems therefore limited[75].

In isolated hepatic perfusion (IHP), drugs are also administered directly into the hepatic artery but in this technique, hepatic circulation is completely shut off from the systemic circulation. Perfusate containing cytostatic drugs (mostly melphalan) is circulated through the liver for a certain period of time, exposing tumour tissue to high drug doses while other vital organs are not affected[76]. With this method, liver tumours can be treated with high doses of chemotherapeutics that would cause severe toxicity if administered systemically[77,78]. Also, other anti-tumour compounds may be added that are not suitable for systemic administration, such as TNF-alpha[79,80]. IHP with varying treatment strategies has resulted in response rates up to 74% and median survival up to 29 months[81-83], and can therefore be of considerable value in the treatment of colorectal liver metastases. It should be noted that the development of new drugs for systemic chemotherapy, such as irinothecan and oxaliplatin, as well as the design of different combination therapies

with several drugs has increased median survival after systemic chemotherapy as well in recent clinical trials[84].

Local ablative techniques and immune response

Various in vivo and in vitro studies have assessed the involvement of the immune system in the efficacy of PDT and indicated an increased specific immune response upon PDT[85-89]. Korbelik *et al.* found that selective depletion of macrophages, neutrophils or T cells significantly inhibited tumour cure of PDT treated sarcomas in mice[90]. In addition, tumour cure after PDT of sarcomas in severe combined immune deficient (SCID) mice was shown to be strongly inhibited. After administration of splenocytes from immune competent mice whose sarcomas had been treated with PDT five weeks earlier, tumour cure was completely restored[91]. Not only is an intact immune system indispensable for an optimal initial antitumour effect of PDT, PDT may also increase resistance to subsequent tumour cell exposure. Chen *et al.* showed that after treatment of rat mammary tumours with PDT, rats did not develop new tumours when rechallenged with the same tumour cell line whereas rats that were treated with resection did develop tumours upon rechallenge[92].

For cryoablation, various in vivo studies into its effect on the immune system have been conducted. As early as 1968, Shulman *et al.* found significantly increased serum titers of auto antibodies in rabbits that underwent cryosurgery of the male accessory glands[93]. Cellular immunity may also play a role after cryotherapy, as rats and mice treated with cryotherapy exhibited increased resistance to subsequent tumour rechallenge[94,95]. Faraci *et al.* published results of an in vivo experiment with mice bearing fibrosarcomas that were treated with a single cryosurgical treatment. After cryotherapy, humoral and cellular toxicity was increased in cryosurgical treated rats when compared to rats treated with resection[96]. The role of cellular immunity upon cryotherapy was further elucidated in two Japanese studies assessing the resistance to tumour rechallenge after cryotherapy of subcutaneous mammary tumours in rats[97,98]. Both authors found that in the first 6 weeks after treatment, tumour resistance was low, but at 10 weeks after treatment tumour resistance increased, exceeding tumour resistance in rats whose initial tumour was treated with surgical excision.

At this moment, only one study concerning the effect of RFA on the immune system has been published. Wissniowski *et al.* showed the presence of circulating tumour specific T cells after RFA treatment of VX2 hepatoma in 11 rabbits as well as increased T cell infiltration in tumour margins after RFA[99]. In addition to these sparse data on RFA and the immune response, two studies concerning LITT have been published[100,101]. Both studies showed that LITT of liver tumours in rats resulted in decreased occurrence of intraperitoneal

tumour spread. In the study by Isbert *et al.*, treatment of one out of two CC531 liver tumours by LITT also inhibited growth of the nearby, untreated tumour.

HAI and IHP differ from these ablative techniques as they are not local but regional, and tumour destruction is not as acute as with the local ablative techniques. At present, there is no data available on the effect of these treatments on the immune system.

If locoregional therapies for colorectal liver metastases indeed cause the generation or enhancement of a tumour specific systemic immune response, this could be a valuable starting point for further improvements in the antitumour efficacy of these techniques [102]. Further research into the exact nature of the interaction between local therapies and the immune system is indispensable and could be the first step towards incorporation of the immunological effects of locoregional therapies for liver metastases in daily clinical practice. It could expand the scope of these treatments from a local to a systemic level, thereby broadening its applicability and possibly improve its clinical results.

Background

In 1999, research into photodynamic therapy for colorectal liver metastases was initiated at the LUMC Department of Surgery. Initially, research was aimed at assessing the possibilities of PDT for colorectal liver metastases using a newly developed photosensitiser. In vivo experiments on pigs and rats proved interstitial PDT with this new sensitiser to be possible and a phase I clinical trial was designed.
This multicentre trial started in 2000 and a total of 24 patients were treated with this technique, 3 of whom in the Department of Surgery of the LUMC. Simultaneously, the RFA technique was introduced in our centre and the first patient was also treated in 2000. In addition to evaluating the antitumour efficacy of these treatments, we formulated the hypothesis that local ablative techniques may induce or enhance a systemic antitumour immune response. In various in vivo experiments on rats, RFA, PDT and HAI and their effects on the immune response were assessed.
This thesis contains results of both the clinical RFA and PDT trials and of the in vivo experiments with these local ablative techniques.

Outline of this thesis

The aims of this thesis were to evaluate the safety and anti-tumour efficacy of local treatments for colorectal liver metastases and to study the effects of these therapies on interaction between tumour cells and immune system

In **Chapter 2**, the difficulty of an immune response to effectively attack established tumours is shown in vivo. As local therapies such as PDT and RFA disrupt the structure of established tumours, they may induce an immune response due to the exposure of tumour antigens to cells of the immune system.
In **Chapters 3** and **4** we assess the effect of PDT, RFA and HAI on the immune system in vivo, using a rat model for colorectal liver metastases. In **Chapter 5**, the occurrence of an immune response following locoregional therapies in patients is evaluated by determination of the level of anti-colorectal carcinoma antibodies and the immune response related cytokines IL-10 and IFN-gamma.
Chapters 6, **7 and 8** report on results from clinical application of local therapies, PDT and RFA. In **Chapter 6**, results of a multicentre phase I trial on PDT for irresectable colorectal liver metastases show PDT to be an effective, safe and feasible treatment option. Adverse effects of the more frequently used RFA is evaluated in a retrospective study on RFA in The Netherlands, which is described in **Chapter 7**. In the following **Chapter 8** we investigate the local efficacy of RFA and identify parameters that influence local failure following RFA.

References

1. Jessup JM, McGinnis LS, Steele GD, Jr., Menck HR, Winchester DP. The National Cancer Data Base. Report on colon cancer. *Cancer* 1996; **78**: 918-26.

2. Weiss L et al. Haematogenous metastatic patterns in colonic carcinoma: an analysis of 1541 necropsies. *J Pathol* 1986; **150**: 195-203.

3. Welch JP, Donaldson GA. The clinical correlation of an autopsy study of recurrent colorectal cancer. *Ann Surg* 1979; **189**: 496-502.

4. Bengmark S, Hafstrom L. The natural history of primary and secondary malignant tumours of the liver. I. The prognosis for patients with hepatic metastases from colonic and rectal carcinoma by laparotomy. *Cancer* 1969; **23**: 198-202.

5. Jaffe BM, Donegan WL, Watson F, Spratt JS, Jr. Factors influencing survival in patients with untreated hepatic metastases. *Surg Gynecol Obstet* 1968; **127**: 1-11.

6. Wood CB, Gillis CR, Blumgart LH. A retrospective study of the natural history of patients with liver metastases from colorectal cancer. *Clin Oncol* 1976; **2**: 285-8.

7. Stangl R, Altendorf-Hofmann A, Charnley RM, Scheele J. Factors influencing the natural history of colorectal liver metastases. *Lancet* 1994; **343**: 1405-10.

8. Silen W. Hepatic resection for metastases from colorectal carcinoma is of dubious value. *Arch Surg* 1989; **124**: 1021-2.

9. Elias D, Ouellet JF, De Baere T, Lasser P, Roche A. Preoperative selective portal vein embolization before hepatectomy for liver metastases: long-term results and impact on survival. *Surgery* 2002; **131**: 294-9.

10. Cady B et al. Surgical margin in hepatic resection for colorectal metastasis: a critical and improvable determinant of outcome. *Ann Surg* 1998; **227**: 566-71.

11. Doci R et al. Morbidity and mortality after hepatic resection of metastases from colorectal cancer. *Br J Surg* 1995; **82**: 377–81.

12. Fong Y et al. Liver resection for colorectal metastases. *J Clin Oncol* 1997; **15**: 938–46.

13. Iwatsuki S et al. Hepatic resection for metastatic colorectal adenocarcinoma: a proposal of a prognostic scoring system. *J Am Coll Surg* 1999; **189**: 291–9.

14. Nordlinger B et al. Surgical resection of colorectal carcinoma metastases to the liver. A prognostic scoring system to improve case selection, based on 1568 patients. Association Francaise de Chirurgie. *Cancer* 1996; **77**: 1254–62.

15. Scheele J, Stang R, Altendorf-Hofmann A, Paul M. Resection of colorectal liver metastases. *World J Surg* 1995; **19**: 59–71.

16. Iwatsuki S et al. Hepatic resection for metastatic colorectal adenocarcinoma: a proposal of a prognostic scoring system. *J Am Coll Surg* 1999; **189**: 291–9.

17. Jamison RL et al. Hepatic resection for metastatic colorectal cancer results in cure for some patients. *Arch Surg* 1997; **132**: 505–10.

18. Nordlinger B et al. Surgical resection of colorectal carcinoma metastases to the liver. A prognostic scoring system to improve case selection, based on 1568 patients. Association Francaise de Chirurgie. *Cancer* 1996; **77**: 1254–62.

19. Scheele J, Stang R, Altendorf-Hofmann A, Paul M. Resection of colorectal liver metastases. *World J Surg* 1995; **19**: 59–71.

20. Fong Y, Fortner J, Sun RL, Brennan MF, Blumgart LH. Clinical score for predicting recurrence after hepatic resection for metastatic colorectal cancer: analysis of 1001 consecutive cases. *Ann Surg* 1999; **230**: 309–18.

21. Hughes KS et al. Resection of the liver for colorectal carcinoma metastases: a multi-institutional study of patterns of recurrence. *Surgery* 1986; **100**: 278-84.

22. Lise M, Bacchetti S, Da Pian P, Nitti D, Pilati P. Patterns of recurrence after resection of colorectal liver metastases: prediction by models of outcome analysis. *World J Surg* 2001; **25**: 638-44.

23. Elias D et al. Resection of liver metastases from colorectal cancer: the real impact of the surgical margin. *Eur J Surg Oncol* 1998; **24**: 174-9.

24. Cady B et al. Surgical margin in hepatic resection for colorectal metastasis: a critical and improvable determinant of outcome. *Ann Surg* 1998; **227**: 566-71.

25. Iwatsuki S et al. Hepatic resection for metastatic colorectal adenocarcinoma: a proposal of a prognostic scoring system. *J Am Coll Surg* 1999; **189**: 291-9.

26. Lise M, Bacchetti S, Da Pian P, Nitti D, Pilati P. Patterns of recurrence after resection of colorectal liver metastases: prediction by models of outcome analysis. *World J Surg* 2001; **25**: 638-44.

27. Steele G, Jr. et al. A prospective evaluation of hepatic resection for colorectal carcinoma metastases to the liver: Gastrointestinal Tumour Study Group Protocol 6584. *J Clin Oncol* 1991; **9**: 1105-12.

28. Cady B et al. Surgical margin in hepatic resection for colorectal metastasis: a critical and improvable determinant of outcome. *Ann Surg* 1998; **227**: 566-71.

29. Fong Y, Fortner J, Sun RL, Brennan MF, Blumgart LH. Clinical score for predicting recurrence after hepatic resection for metastatic colorectal cancer: analysis of 1001 consecutive cases. *Ann Surg* 1999; **230**: 309-18.

30. Iwatsuki S et al. Hepatic resection for metastatic colorectal adenocarcinoma: a proposal of a prognostic scoring system. *J Am Coll Surg* 1999; **189**: 291-9.

31. Lise M, Bacchetti S, Da Pian P, Nitti D, Pilati P. Patterns of recurrence after resection of colorectal liver metastases: prediction by models of outcome analysis. *World J Surg* 2001; **25**: 638-44.

32. Nordlinger B et al. Surgical resection of colorectal carcinoma metastases to the liver. A prognostic scoring system to improve case selection, based on 1568 patients. Association Francaise de Chirurgie. *Cancer* 1996; **77**: 1254-62.

33. Scheele J, Stang R, Altendorf-Hofmann A, Paul M. Resection of colorectal liver metastases. *World J Surg* 1995; **19**: 59-71.

34. Bilchik AJ et al. Radiofrequency ablation: a minimally invasive technique with multiple applications. *Cancer J Sci Am* 1999; **5**: 356-61.

35. De Baere, T., Elias, D., Ducreux, M., Kuoch, V., Boige, V., Dromain, C., Lasser, P., and Tursz, T. Percutaneous radiofrequency of liver metastases: Single center experience over a five year period. Proc Am Soc Clin Oncol 1387, 345. 2003.

36. Jiao LR et al. Clinical short-term results of radiofrequency ablation in primary and secondary liver tumours. *Am J Surg* 1999; **177**: 303-6.

37. Oshowo A, Gillams A, Harrison E, Lees WR, Taylor I. Comparison of resection and radiofrequency ablation for treatment of solitary colorectal liver metastases. *Br J Surg* 2003; **90**: 1240-3.

38. Parikh AA, Curley SA, Fornage BD, Ellis LM. Radiofrequency ablation of hepatic metastases. *Semin Oncol* 2002; **29**: 168-82.

39. Scaife CL, Curley SA. Complication, local recurrence, and survival rates after radiofrequency ablation for hepatic malignancies. *Surg Oncol Clin N Am* 2003; **12**: 243-55.

40. Solbiati L et al. Percutaneous radio-frequency ablation of hepatic metastases from colorectal cancer: long-term results in 117 patients. *Radiology* 2001; **221**: 159-66.

41. Liu LX, Zhang WH, Jiang HC. Current treatment for liver metastases from colorectal cancer. *World J Gastroenterol* 2003; **9**: 193-200.

42. Neeleman N, Wobbes T, Jager GJ, Ruers TJ. Cryosurgery as treatment modality for colorectal liver metastases. *Hepatogastroenterology* 2001; **48**: 325-9.

43. Ruers TJ, Joosten J, Jager GJ, Wobbes T. Long-term results of treating hepatic colorectal metastases with cryosurgery. *Br J Surg* 2001; **88**: 844-9.

44. Sheen AJ, Poston GJ, Sherlock DJ. Cryotherapeutic ablation of liver tumours. *Br J Surg* 2002; **89**: 1396-401.

45. Sotsky TK, Ravikumar TS. Cryotherapy in the treatment of liver metastases from colorectal cancer. *Semin Oncol* 2002; **29**: 183-91.

46. Adam R et al. A comparison of percutaneous cryosurgery and percutaneous radiofrequency for unresectable hepatic malignancies. *Arch Surg* 2002; **137**: 1332-9.

47. Tait IS, Yong SM, Cuschieri SA. Laparoscopic in situ ablation of liver cancer with cryotherapy and radiofrequency ablation. *Br J Surg* 2002; **89**: 1613-9.

48. Izzo F. Other thermal ablation techniques: microwave and interstitial laser ablation of liver tumours. *Ann Surg Oncol* 2003; **10**: 491-7.

49. Gillams AR, Lees WR. Survival after percutaneous, image-guided, thermal ablation of hepatic metastases from colorectal cancer. *Dis Colon Rectum* 2000; **43**: 656-61.

50. Mack MG et al. Percutaneous MR imaging-guided laser-induced thermotherapy of hepatic metastases. *Abdom Imaging* 2001; **26**: 369-74.

51. Vogl TJ, Mack MG, Straub R, Roggan A, Felix R. Magnetic resonance imaging--guided abdominal interventional radiology: laser-induced thermotherapy of liver metastases. *Endoscopy* 1997; **29**: 577-83.

52. Vogl TJ, Straub R, Eichler K, Sollner O, Mack MG. Colorectal carcinoma metastases in liver: laser-induced interstitial thermotherapy--local tumour control rate and survival data. *Radiology* 2004; **230**: 450-8.

53. Boyle RW, Dolphin D. Structure and biodistribution relationships of photodynamic sensitizers. *Photochem Photobiol* 1996; **64**: 469-85.

54. Bugelski PJ, Porter CW, Dougherty TJ. Autoradiographic distribution of hematoporphyrin derivative in normal and tumour tissue of the mouse. *Cancer Res* 1981; **41**: 4606-12.

55. Fingar VH. Vascular effects of photodynamic therapy. *J Clin Laser Med Surg* 1996; **14**: 323-8.

56. Rovers JP, Schuitmaker JJ, Vahrmeijer AL, van Dierendonck JH, Terpstra OT. Interstitial photodynamic therapy with the second-generation photosensitizer bacteriochlorin a in a rat model for liver metastases. *Br J Cancer* 1998; **77**: 2098-103.

57. Rovers JP et al. Effective treatment of liver metastases with photodynamic therapy, using the second-generation photosensitizer meta- tetra(hydroxyphenyl)chlorin (mTHPC), in a rat model. *Br J Cancer* 1999; **81**: 600-8.

58. van Duijnhoven FH, Rovers JP, Terpstra OT. Photodynamic therapy with mTHPBC for colorectal liver metastases is safe and feasible: results from a phase I study. *Cancer Res* 2004.

59. Livraghi T et al. Hepatocellular carcinoma and cirrhosis in 746 patients: long-term results of percutaneous ethanol injection. *Radiology* 1995; **197**: 101-8.

60. Giovannini M, Seitz JF. Ultrasound-guided percutaneous alcohol injection of small liver metastases. Results in 40 patients. *Cancer* 1994; **73**: 294-7.

61. Livraghi T, Vettori C, Lazzaroni S. Liver metastases: results of percutaneous ethanol injection in 14 patients. *Radiology* 1991; **179**: 709-12.

62. Amin Z, Bown SG, Lees WR. Local treatment of colorectal liver metastases: a comparison of interstitial laser photocoagulation (ILP) and percutaneous alcohol injection (PAI). *Clin Radiol* 1993; **48**: 166-71.

63. Isbert C et al. In situ ablation of experimental liver metastases delays and reduces residual intrahepatic tumour growth and peritoneal tumour spread compared with hepatic resection. *Br J Surg* 2002; **89**: 1252-9.

64. Puls R et al. Laser-induced thermotherapy (LITT) of liver metastases: MR-guided percutaneous insertion of an MRI-compatible irrigated microcatheter system using a closed high-field unit. *J Magn Reson Imaging* 2003; **17**: 663-70.

65. Blomgren H, Lax I, Naslund I, Svanstrom R. Stereotactic high dose fraction radiation therapy of extracranial tumours using an accelerator. Clinical experience of the first thirty-one patients. *Acta Oncol* 1995; **34**: 861-70.

66. Robertson JM et al. The treatment of colorectal liver metastases with conformal radiation therapy and regional chemotherapy. *Int J Radiat Oncol Biol Phys* 1995; **32**: 445-50.

67. Herfarth KK et al. Stereotactic single-dose radiation therapy of liver tumours: results of a phase I/II trial. *J Clin Oncol* 2001; **19**: 164-70.

68. Sigurdson ER, Ridge JA, Kemeny N, Daly JM. Tumour and liver drug uptake following hepatic artery and portal vein infusion. *J Clin Oncol* 1987; **5**: 1836-40.

69. Wang LQ, Persson BG, Stenram U, Bengmark S. Influence of portal branch ligation on the outcome of repeat dearterializations of an experimental liver tumour in the rat. *J Surg Oncol* 1994; **55**: 229-34.

70. Kemeny N et al. Hepatic arterial infusion of chemotherapy after resection of hepatic metastases from colorectal cancer. *N Engl J Med* 1999; **341**: 2039-48.

71. Kemeny N et al. Phase I study of hepatic arterial infusion of floxuridine and dexamethasone with systemic irinotecan for unresectable hepatic metastases from colorectal cancer. *J Clin Oncol* 2001; **19**: 2687-95.

72. Kemeny N et al. Intrahepatic or systemic infusion of fluorodeoxyuridine in patients with liver metastases from colorectal carcinoma. A randomized trial. *Ann Intern Med* 1987; **107**: 459-65.

73. Lorenz M, Muller HH. Randomized, multicenter trial of fluorouracil plus leucovorin administered either via hepatic arterial or intravenous infusion versus fluorodeoxyuridine administered via hepatic arterial infusion in patients with nonresectable liver metastases from colorectal carcinoma. *J Clin Oncol* 2000; **18**: 243-54.

74. Vahrmeijer AL, van Dierendonck JH, van de Velde CJ. Treatment of colorectal cancer metastases confined to the liver. *Eur J Cancer* 1995; **31A**: 1238-42.

75. Meta-Analysis Group in Cancer. Reappraisal of hepatic arterial infusion in the treatment of nonresectable liver metastases from colorectal cancer. *J Natl Cancer Inst* 1996; **88**: 252-8.

76. Vahrmeijer AL 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**: 1539-46.

77. Marinelli A 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**: 341-50.

78. Vahrmeijer AL, van Dierendonck JH, van de Velde CJ. Treatment of colorectal cancer metastases confined to the liver. *Eur J Cancer* 1995; **31A**: 1238-42.

79. Alexander HR et al. A phase I-II study of isolated hepatic perfusion using melphalan with or without tumour necrosis factor for patients with ocular melanoma metastatic to liver. *Clin Cancer Res* 2000; **6**: 3062-70.

80. Alexander HR, Jr. et al. Isolated hepatic perfusion with tumour necrosis factor and melphalan for unresectable cancers confined to the liver. *J Clin Oncol* 1998; **16**: 1479-89.

81. Alexander HR, Jr. et al. Isolated hepatic perfusion with tumour necrosis factor and melphalan for unresectable cancers confined to the liver. *J Clin Oncol* 1998; **16**: 1479-89.

82. Bartlett DL, Libutti SK, Figg WD, Fraker DL, Alexander HR. Isolated hepatic perfusion for unresectable hepatic metastases from colorectal cancer. *Surgery* 2001; **129**: 176-87.

83. Rothbarth J et al. Isolated hepatic perfusion with high-dose melphalan for the treatment of colorectal metastasis confined to the liver. *Br J Surg* 2003; **90**: 1391-7.

84. Tournigand C et al. FOLFIRI Followed by FOLFOX6 or the Reverse Sequence in Advanced Colorectal Cancer: A Randomized GERCOR Study. *J Clin Oncol* 2003.

85. Chen WR, Zhu WG, Dynlacht JR, Liu H, Nordquist RE. Long-term tumour resistance induced by laser photo-immunotherapy. *Int J Cancer* 1999; **81**: 808-12.

86. Coutier S et al. Foscan (mTHPC) photosensitized macrophage activation: enhancement of phagocytosis, nitric oxide release and tumour necrosis factor-alpha- mediated cytolytic activity. *Br J Cancer* 1999; **81**: 37-42.

87. Korbelik M. Induction of tumour immunity by photodynamic therapy. *J Clin Laser Med Surg* 1996; **14**: 329-34.

88. van Duijnhoven FH, Aalbers RI, Rovers JP, Terpstra OT, Kuppen PJ. Immunological aspects of photodynamic therapy of liver tumours in a rat model for colorectal cancer. *Photochem Photobiol* 2003; **78**: 235-40.

89. van Duijnhoven, F. H., Tollenaar, R. A., Terpstra, O. T., and Kuppen, P. J. Locoregional therapies of liver metastases in a rat CC531 coloncarcinoma model have an effect on the anti-tumour immune response. 2004.

90. Korbelik M, Cecic I. Contribution of myeloid and lymphoid host cells to the curative outcome of mouse sarcoma treatment by photodynamic therapy. *Cancer Lett* 1999; **137**: 91-8.

91. Korbelik M, Cecic I. Contribution of myeloid and lymphoid host cells to the curative outcome of mouse sarcoma treatment by photodynamic therapy. *Cancer Lett* 1999; **137**: 91-8.

92. Chen WR, Zhu WG, Dynlacht JR, Liu H, Nordquist RE. Long-term tumour resistance induced by laser photo-immunotherapy. *Int J Cancer* 1999; **81**: 808-12.

93. Shulman S, Brandt EJ, Yantorno C. Studies in cryo-immunology. II. Tissue and species specificity of the autoantibody response and comparison with iso-immunization. *Immunology* 1968; **14**: 149-58.

94. Neel HB, III, Ketcham AS, Hammond WG. Experimental evaluation of in situ oncocide for primary tumour therapy: comparison of tumour-specific immunity after complete excision, cryonecrosis and ligation. *Laryngoscope* 1973; **83**: 376-87.

95. Blackwood CE, Cooper IS. Response of experimental tumour systems to cryosurgery. *Cryobiology* 1972; **9**: 508-15.

96. Faraci RP, Bagley DH, Marrone J, Beazley RM. In vitro demonstration of cryosurgical augmentation of tumour immunity. *Surgery* 1975; **77**: 433-8.

97. Misao A, Sakata K, Saji S, Kunieda T. Late appearance of resistance to tumour rechallenge following cryosurgery. A study in an experimental mammary tumour of the rat. *Cryobiology* 1981; **18**: 386-9.

98. Miya K et al. Immunological response of regional lymph nodes after tumour cryosurgery: experimental study in rats. *Cryobiology* 1986; **23**: 290-5.

99. Wissniowski TT et al. Activation of tumour-specific T lymphocytes by radio-frequency ablation of the VX2 hepatoma in rabbits. *Cancer Res* 2003; **63**: 6496-500.

100. Isbert C et al. In situ ablation of experimental liver metastases delays and reduces residual intrahepatic tumour growth and peritoneal tumour spread compared with hepatic resection. *Br J Surg* 2002; **89**: 1252-9.

101. Moller PH, Ivarsson K, Stenram U, Radnell M, Tranberg KG. Comparison between interstitial laser thermotherapy and excision of an adenocarcinoma transplanted into rat liver. *Br J Cancer* 1998; **77**: 1884-92.

102. Gollnick SO, Vaughan L, Henderson BW. Generation of effective antitumour vaccines using photodynamic therapy. *Cancer Res* 2002; **62**: 1604-8.