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Faculteit Geneeskunde en Gezondheidswetenschappen
Vakgroep Experimentele Cancerologie, Radiotherapie en Kerngeneeskunde

New Strategies in Radionuclide Therapy for Hepatocellular Carcinoma

Bieke Lambert

Proefschrift ter verkrijging van het doctoraat in de Medische Wetenschappen
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Introduction

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General principles of radionuclide therapy

Radionuclide therapy may be defined as internal radiotherapy by means of unsealed sources of ionizing radiation. It typically targets particular organs or cells. The targeting feature of radionuclide therapy is achieved by local or locoregional administration of the radionuclide, by the intrinsic targeting properties of some radionuclides or by conjugating the radionuclide to a specific tracer. The radionuclides used in routine practice for therapeutic applications are beta-particle emitters. These β-particles consist of high energy electrons exerting ionizing effects in the targeted tissue. Ionizing radiation damages the DNA of the targeted cells and thus lethal damage may possibly be induced on a cellular level.
The range of the electrons depends on the energy of the emitted β-particles of the applied radionuclide and for high energy β-emitters this range surpasses multiple cell diameters. Besides β-emissions, some radionuclides also emit photons or gamma (γ)-rays which are detectable by means of a γ–camera and hence allow for imaging the distribution of the radionuclide in the patient. However, photons are penetrating ionizing radiation, and as a consequence, treatment may entail considerable radiation protection concerns for the patient's environment.
The physical half-life determines the characteristic rate at which decay occurs for a particular radionuclide. The physical life of radionuclides used for internal radiotherapy varies widely but compared to external beam radiotherapy, radionuclide therapies are considered as low dose rate irradiation because the rate at which the radionuclides deposit their energy to the targeted cells is prolonged over time whereas external beam irradiation delivers a high dose in a very short time interval, typically a few Gy in a few minutes.

Besides its merits in the management of benign disorders, such as thyroid disease and chronic joint inflammation, radionuclide therapy has shown its usefulness in oncology, for instance in the management of thyroid cancer, metastatic neuro-endocrine tumors, bone pain palliation and locoregional treatment for primary liver cancer or liver metastasis. More recently, promising progress was established in the field of hematology. However, radionuclide therapy remains a relatively unknown treatment modality for many colleagues, even those working in the fields of oncology. Despite the available literature reporting the clinical benefits of radiolabeled diphosphonates, meta-iodobenzylguanidine, somatostatin analogues and antibodies, the application of these radiopharmaceuticals remains limited to few expert centers in well developed countries. Radioprotection concerns, limited availability and high costs of some radionuclides as well as compounds, such as monoclonal antibodies and peptides, have been drawbacks for widespread implementation of radionuclide based treatment strategies.

Rhenium-188 for radionuclide therapy

Similar to the introduction of the in-house use of the Molybdenum/Technetium (^{99}Mo/^{99m}Tc)-generator in a radiopharmacy for the radiolabeling of a wide variety of diagnostic agents, generator derived therapeutic isotopes would contribute to the further development and implementation of radionuclide therapies. High activities of Rhenium-188 (^{188}Re) are available from the elution on an on-site installable Tungsten/Rhenium (^{188}W/^{188}Re)-generator.

^{188}Re is of widespread interest for therapeutic applications because of its high energy β-emission (maximum energy 2.12 MeV). Its γ-emission of 155 keV with an abundance of 15% and relatively short half-life of 16.9 hours, limit radioprotection problems and represent a firm advantage in comparison with the nowadays widespread used Iodine-131 (^{131}I) for various therapeutic radiopharmaceuticals (Table 1). The γ-rays emitted by ^{188}Re allow imaging of the distribution of the radionuclide in the patient. If the patient is scanned at several timepoints, the absorbed doses to the organs may be estimated following quantitative analysis.

In contrast to ^{131}I the use of ^{188}Re does not pose significant radiation protection problems since the energy and abundance of the γ-emissions is lower and the physical half-life is shorter. Hence the use of ^{188}Re is more convenient than ^{131}I for routine clinical use due to the relative ease of waste management, the decreased radiation burden for hospital staff and patient's relatives and the shorter duration that a patient must remain in a dedicated radionuclide therapy suite. In addition, the flexibility of the treatment planning is improved due to the shorter hospitalization and on-site production of the radiopharmaceutical.

TABLE 1: Physical characteristics of Iodine-131 and Rhenium-188.

Characteristic	**Iodine-131**	**Rhenium-188**
Eγ (abundance)	364 keV (81.7%)	155 keV (15.0%)
Eβ max (abundance)	606 keV (89.9%)	2120 keV (71.1%), 1960 keV (25.6%)
physical half-life	8.01 days	16.9 hours
production	fission product	generator

^{188}Re is obtained on demand as carrier-free sodium perrhenate by saline elution of the ^{188}W/^{188}Re-generator system. The ^{188}W-parent is produced by double neutron capture of enriched ^{186}W-oxide targets by irradiation in a nuclear reactor. Following various dissolving and acidification steps, the ^{188}W-parent is subsequently adsorbed on an alumia column. ^{188}W has a physical half-life of 69 days. Generators designed for clinical use are typically loaded with 37-55.5 GBq ^{188}W and provide about 18.5 GBq ^{188}Re-perrhenate on a daily basis for at least 8 weeks. The ^{188}W/^{188}Re-generator system operates similarly to the ^{99}Mo/^{99m}Tc-generator (*1*).

Hepatocellular carcinoma

Hepatocellular carcinoma (HCC) is a malignant epithelial tumor arising from parenchymatous liver cells (*2*). It is the most prevalent primary liver cancer. In terms of numbers of cases, HCC is the sixth most common cancer worldwide with 626.000 new cases estimated for the year 2002. Because of the very poor prognosis, the number of deaths (598.000) is almost equal to the number of new cases per year. It is therefore the third most common cause of death from cancer. The vast majority of new cases occur in developing countries (82%). The areas of high incidence are sub-Saharan Africa, eastern and southeastern Asia, and Melanesia. The incidence is low in Latin America, southcentral Asia and in developed areas. Only in southern Europe is there any substantial risk. An overview of incidence rates of HCC in various regions of the world is given in Table 2. The overall sex ratio (male:female) is around 2.4 (*2*).

TABLE 2: Age-standardized incidence rates for primary liver cancer. Data shown per 100.000 by sex. Table adapted from Parkin DM et al. *CA Cancer J Clin* 2005;55:74-108.

	Males	**Females**
China	37.9	14.2
Middle Africa	27.8	13.4
Japan	23.1	7.6
Southern Europe	11.6	4.0
Western Europe	6.2	1.7
Eastern Europe	5.3	2.4
Northern Europe	3.4	1.7
Northern America	5.3	1.9

The major risk factor for HCC is cirrhosis. All types of cirrhosis predispose to HCC, but the incidence is particularly high in persistent infection with hepatitis B (HBV) and hepatitis C (HCV), and in alcoholic liver disease. In Asia and Africa chronic infection by the hepatitis B virus is the main risk factor for development of HCC, whereas the hepatitis C virus and alcohol are relatively more important in the Western world and Japan (*4*). Although the overall incidence of HCC in the West is low compared to South East Asia, Sub-Saharan Africa and Japan, its incidence is rising (*5,6*).

The awareness of the association between cirrhosis and HCC has resulted in the widespread use of surveillance schemes including ultrasound and serum alpha-fetoprotein (AFP) measurements (*7*). The tumor marker AFP is a protein normally made by the immature liver cells in the fetus. In adults suffering chronic hepatitis or cirrhosis moderate levels of AFP can be seen. Blood levels over 400 ng/ml of AFP are suggestive for HCC. Additional, size-dependent examinations (*8,9*) are performed when confronted with a nodular lesion emerging in a cirrhotic liver in order to assess the presence of HCC and its intrahepatic spread. In nodules smaller than 2 cm, fine-needle biopsy may play

a role in establishing the diagnosis of HCC. However, since high false-negative rates as well as tumoral seeding following fine needle biopsies have been reported, close follow-up by means of repeated imaging is often recommended whenever a potentially curative lesion is present (*10,11*). In nodules larger than 2 cm, HCC diagnosis is established by the concomitant finding of two imaging techniques, showing a nodule larger than 2 cm with arterial hypervascularization, or by one positive imaging technique, showing hypervascularization associated with AFP concentration higher than 400 ng/ml (*11*). The best imaging techniques are spiral computed tomography (CT-scan) and magnetic resonance imaging (MRI) with contrast enhancement, which have an accuracy exceeding 80% (*9*). For assessment of the extent of the liver involvement by HCC, the sensitivity and specificity of both MRI and CT is markedly lower (*8,9*). Extrahepatic spread is ruled out by spiral CT-scan of the chest and by bone scintigraphy (*11*) .

The selection of an appropriate treatment strategy for patients with HCC depends on careful tumor staging and assessment of the underlying liver disease (*12*). All patients without vascular invasion or extrahepatic disease should be evaluated for the potentially curative therapeutic options of partial hepatectomy or orthotopic liver transplantation. According to the so called Milan criteria, orthotopic liver transplantation should be considered if there is a solitary lesion smaller than 5 cm in diameter or fewer than 3 lesions smaller than 3 cm (*13*). Five-year survival following partial hepatectomy is on average 25-45% and when considering highly-selected patient groups as high as 70% (*12*). Local ablative therapies such as Percutaneous Ethanol Injection (PEI), Radio Frequency Ablation (RFA), and Trans Arterial Chemo Embolization (TACE) offer palliation for patients for whom surgical approaches are contraindicated (*11*). PEI and RFA are minimally invasive and can be used on an outpatient basis, usually for tumor nodules smaller than 3 cm for PEI and up to 5 cm for RFA (*14*). When these therapies are used for small tumors, survival rates obtained from prospective cohort studies and retrospective analysis, show similar results as those achieved by partial hepatectomy (*16-17*). The precise role of TACE in a palliative setting as well in patients awaiting liver transplantation is still under debate but encouraging long term results have been obtained in randomized controlled trials (*18*). Whilst PEI, RFA and TACE are useful in selected patients, they are not suitable for many HCC patients with advanced disease. For patients presenting with involvement of regional lymph-nodes, extra-hepatic metastases or involvement of the main portal vein, systemic treatment is an option (*19-21*). However, no strong evidence is provided that any systemic chemotherapy, hormonal therapy, or immunotherapy regimen studied to date benefits survival of HCC patients in this setting (*22-24*). External beam irradiation resulted in palliation of symptoms in more than half of the patients in some series but injury to the surrounding tissue including liver parenchyma and duodenum is risked (*25-29*).

Radiolabeled Lipiodol for treatment of hepatocellular carcinoma

Lipiodol consists of a mixture of mono-, di- and tri-iodinated ethyl esters of mainly linoleic, oleic and palmitic acid. It is manufactured by ethyl trans-esterification of poppyseed oil (Laboratoire Guerbet, France) and contains 37-39 % of iodine by weight. It has been used as a contrast material for the detection of HCC. When injected into the hepatic artery the oil is retained by HCCs for several weeks to over a year, but it is cleared from the normal liver parenchyma within 7 days (*30,31*). Several mechanisms explaining this selective retention have been postulated and hypotheses fall into three major categories. The first hypothesis mainly suggests that the Lipiodol is retained in the blood vessels. This could be explained by the embolization effects of the Lipiodol droplets and/or the presence of abnormal tumor vessels, possibly with altered electrostatic charge on the endothelial surface facilitating lipid adsorption. A second hypothesis proposes the lodging of Lipiodol in the extra-cellular space. This could be explained by the presence of 'leaky' tumor vessels and a lack of macrophages or lymphatics in the tumor. A third hypothesis suggests the incorporation of Lipiodol in the tumoral cells. *In vitro* experiments with a human HCC cell line have shown rapid active uptake of Lipidol by HCC cells through pinocytosis (*31*). Whilst Lipiodol alone does not appear to have any significant anti-cancer effect, adding a radionuclide to Lipiodol has proven to be effective against HCC *in vitro* (*32*).

^{131}I-Lipiodol for palliative treatment of HCC

Sixteen original papers have been published on the palliative use of ^{131}I-Lipiodol in HCC patients. These papers reported on results obtained in 1 to 73 HCC patients treated with ^{131}I-Lipiodol receiving a wide range of radioactivity (74 to 6220 MBq) in single or multiple administrations. Using either a drop in AFP levels or a reduction in tumor size as response criterion, reported response rates varied from 17 to 92% (*33-35*). Palliative treatment with ^{131}I-Lipiodol (n=65 patients) was compared to TACE (n=64 patients) in a prospective, randomized trial and survival after 6 months, 1, 2, 3 and 4 years were respectively 69%, 38%, 22%, 14% and 10% for the ^{131}I-Lipiodol arm versus 66%, 42%, 22%, 3% and 0% in the TACE-arm. Importantly, however, treatment tolerance was much better in the ^{131}I-Lipiodol arm with only 3 reported serious side-effects versus 29 in the group undergoing TACE (*36*). These results are in line with an earlier report by Bhattacharya et al. (*37*).

While no survival benefit could be demonstrated for the whole group of palliative HCC patients, when focusing on HCC patients presenting with portal vein thrombosis a significant benefit in survival following ^{131}I-Lipiodol treatment versus tamoxifen, 5-fluorouracil or anti-inflammatory medication was documented in a prospective randomized study by Raoul and coworkers (*38*).

^{131}I-Lipidol for adjuvant treatment of HCC

Recurrence rates following partial liver resection are high and this is explained by the often multifocal nature of HCC: the remaining liver parenchyma may harbor metastases that are undetected by currently available imaging techniques and if cirrhotic, it is a seed-bed for development of de novo HCC. In a study by Lau et al. patients undergoing curative resection for HCC and recovering within 6 weeks, were randomly assigned one 1850 MBq administration of ^{131}I-Lipiodol (n = 21) or no further treatment (n= 22). During a median follow-up of 35 months, there were significantly less recurrences among the patients in the adjuvant treatment (28.5%), compared with the control group (59%). Median disease-free survival in the treatment and control groups was 57 and 14 months (*39*). The statistical methodology used for data analysis by these authors, however, was criticized and a longer follow-up advocated by Pocock et al., also stressing the need for additional prospective studies (*39*). Non-randomized or retrospective studies by Partensky et al. and Raoul and coworkers tend to support the findings by Lau et al. (*39,41-43*).

^{131}I-Lipiodol in a neoadjuvant setting

In a preliminary, prospective study by Brans et al., 10 consecutive HCC patients were treated by intra-arterial injection of ^{131}I-Lipiodol into the hepatic artery followed by liver transplantation within 1-9 months. In 5 of the patients studied, an objective response was seen whereas all 10 patients were subsequently transplanted successfully (*44*). Raoul et al. studied 14 patients treated with two sessions of ^{131}I-Lipiodol prior to liver transplantation and 1- and 3 year recurrence free survival rates of 91 and 83% were documented (*42*). Larger studies are mandatory and the long term results should be based on an intention to treat analysis and the true role of neoadjuvant treatment may be preventing drop-out in those patients who wait for long periods until transplantation (*45*).

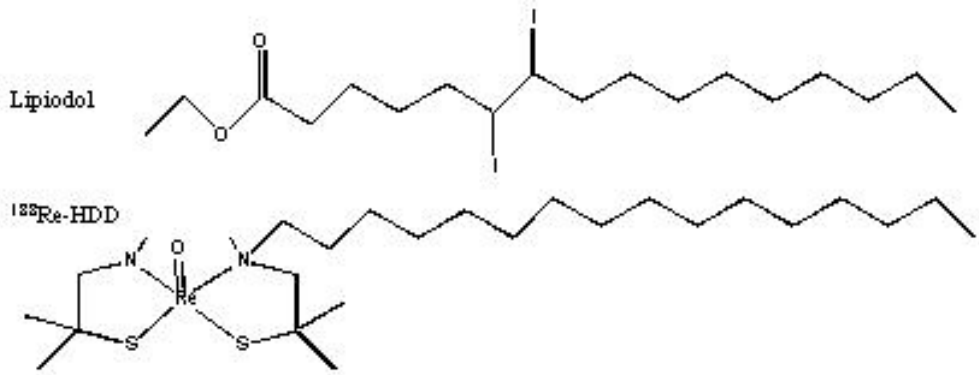
^{188}Re labeled Lipiodol

Encouraging results have been obtained using ^{131}I-Lipiodol, but the use of the radionuclide ^{131}I has hampered its routine clinical implementation. The relatively long physical half-life of ^{131}I and the high energy γ-ray necessitate prolonged hospitalization for radioprotection purposes. Although ^{131}I-Lipiodol therapy is generally well tolerated, a dose escalation study was not conducted so far as the administration of activities exceeding 2.22 GBq is restricted by the above mentioned practical issues. ^{188}Re has favorable characteristics for radionuclide therapy and considering the limited success of ^{131}I-Lipiodol for treatment of relatively large tumors, the switch towards a radionuclide with a higher energy of the beta-emission might yield improved response rates (*34*). The γ-emissions of ^{188}Re, which are distinctly lower in energy and abundance (155 keV, 15.0%) than the γ-emissions from ^{131}I (364 keV, 81.7%), allow for imaging and dosimetry. In contrast to ^{131}I however, the use of ^{188}Re does not imply

significant radiation protection problems since the energy and abundance of the γ-emissions is lower and the physical half-life is shorter.

During the past ten years, promising preclinical results using ^{188}Re labeled Lipiodol for treatment of HCC have been reported (*46-49*). Wang et al. analyzed the biodistribution of ^{188}Re-EDTB-Lipiodol after intrahepatic arterial injection in hepatic tumor bearing rats. The biological half-life of ^{188}Re-EDTB-Lipiodol in healthy liver tissue was 34 hours versus 123 hours in hepatic tumors (*46,47*) but the radiolabeling procedure was elaborative. Jeong and coworkers from the Seoul National University synthesized long-chain alkyl DD (diaminedithiol) derivatives and animal experiments showed that with increasing length of the alkyl chain, tissue uptake and retention improved due to hydrophobic interaction with Lipiodol. Among several synthesized compounds, the Lipiodol solution of ^{188}Re-HDD (4-hexadecyl-1-2,9,9-tetramethyl-4,7-diaza-1,10-decanethiol, Figure 1) had the most favorable tumor retention properties and dosimetric characteristics (*49*).

FIGURE 1: Molecular structure of Lipiodol and the chelating agent HDD. The depiction of Lipiodol is a presumed structure. Adapted from Lee YS et al. *Nucl Med Commun* 2002;23:237-242.



Multimodality treatment based on radiolabeled Lipiodol therapy

A combination of treatment modalities could result in improvement of outcome without induction of severe toxicity (*50*). Regimens combining systemic chemotherapy and external beam radiotherapy were introduced into clinical routine for a variety of tumors. Due to the complex interactions between ionizing radiation and the cytotoxic drugs, the sequence of each modality is important. Clinical results of induction chemotherapy followed by external beam radiotherapy did not yield improvements in local control rates of radiotherapy so far. On the other hand, evidence is available that concurrent chemo-radiotherapy results in improved local control and survival (*51*). The combined use of external beam radiotherapy and cytostatic drugs such as cisplatin, 5-fluorouracil, gemcitabin, or topoisomerase inhibitors has shown promising results for treatment of non-small cell lung cancer, colorectal cancer, pancreatic cancer, head and neck tumors, and a variety of other tumors (*52*).

Concerning intensification of treatment for HCC, phase I clinical studies are being conducted to investigate the benefit of adding systemic or intra-arterial chemotherapy to locoregional irradiation by ^{131}I or ^{188}Re labeled Lipiodol and 5-fluorouracil and cisplatin were used with concurrent external beam irradiation (*53-55*). Of particular interest at present are Lipiodolized emulsions that remain in the tumor for long periods and release drugs in a sustained pattern. A wide variety of cytostatic drugs have been mixed successfully in Lipiodol which can also be radiolabeled with ^{131}I or ^{188}Re (*56-58*).

Enhancement of the effects of ionizing radiation by chemotherapy, is assumed to be related to (1) direct enhancement of the initial radiation damage by incorporating drugs into DNA, (2) inhibiting cellular repair, (3) accumulating cells in a radiosensitive phase or eliminating radioresistant phase cells, (4) eliminating hypoxic cells, (5) inhibiting the accelerated repopulation of tumor cells (*59*).

In the present work the radiosensitizing effects of a number of cytostatic drugs that have been tested in monotherapy for HCC, were investigated using an *in vitro* model for HCC. The set of drugs tested consisted of cisplatinum, 5-fluorouracil, gemcitabin and topotecan. The first two drugs have been widely applied for systemic or locoregional chemotherapy in case of inoperable HCC (*20*). Gemcitabine and topotecan were introduced more recently and so far, only phase I or II studies evaluating its clinical use for advanced HCC are available (*60-62*). The occurrence of supra-additive effects was studied in respect to the type of ionizing radiation applied: a comparison was made between external beam irradiation and exposure to ^{188}Re.

Outline of the thesis

The objective of this work was to explore various new strategies for radionuclide therapy of HCC.
The first part of the clinical research consists of 3 prospective studies concerning the implementation of ^{188}Re labeled Lipiodol for treatment of HCC. In the initial study, the biodistribution, dosimetry and safety aspects following the intra-arterial injection of 3.6 GBq of the new radioconjugate, ^{188}Re-HDD/Lipiodol, were described in detail. In patients with a well compensated underlying cirrhosis, increasing activities were administered and the effects on the liver and lung parameters were carefully monitored. In a third study, attention was paid to its tolerance in patients suffering HCC and moderately advanced cirrhosis.
The second part of the clinical research focuses on patients treated with radiolabeled Lipiodol while awaiting liver transplantation. A retrospective analysis of the anti-tumoral effects, drop-out rate from the waiting list and clinical outcome was conducted.

The second part of this thesis focuses on strategies for optimization of radionuclide therapy of HCC by combining it with cytotoxic drugs with radiosensitizing features. The aim of this preclinical work was to screen cytotoxic drugs, relevant in the treatment of HCC, for enhancement of the effects of irradiation in an *in vitro* model for HCC. In an initial study 3 chemotherapeutic agents were tested for supra-additive effects if combined with external beam irradiation. In a subsequent study the occurrence of supra-additive effects was studied in respect to the type of ionizing radiation applied: a comparison was made between external beam irradiation and exposure to ^{188}Re.

References

1. Knapp FF. Rhenium-188- A generator-derived radioisotope for cancer therapy. *Cancer Biother Radiopharm* 1998;13:337-349.
2. Röcken C, Carll-McGrath S. Pathology and Pathogenesis of Hepatocellular Carcinoma. *Dig Dis* 2001;19:269-278.
3. Parkin DM, Bray F, Ferlay J and Pisani P. Global Cancer Statistics, 2002. *CA Cancer J Clin* 2005;55:74-108.
4. Monto A, Wright TL. The Epidemiology and Prevention of Hepatocellular Carcinoma. *Semin Oncol* 2001;28:441-449.
5. El-Serag HB, Masson AC. Rising incidence of hepatocellular carcinoma in the United States. *N Eng J Med* 1999;340:745-750.
6. El-Serag HB, Davila JA, Petersen NJ, McGlynn KA. The continuing increase in the incidence of hepatocellular carcinoma in the United States: an update. *Ann Intern Med* 2003;139:817-823.
7. Sherman M. Surveillance for hepatocellular carcinoma. *Semin Oncol* 2001;28:450-459.
8. Ward J, Robinson PJ. How to detect hepatocellular carcinoma in cirrhosis. *Eur Radiol* 2002;12:2258-2272.
9. Coakley FV, Schwartz LH. Imaging of hepatocellular carcinima: A practical approach. *Semin Oncol* 2001;28:460-473.
10. Caturelli E, Ghittoni G, Roselli P, De Palo M, Anti M. Fine needle biopsy of focal liver lesions: the hepatologist's point of view. *Liver Transpl* 2004;10:S26-S29.
11. Bruix J, Sherman M, Llovet JM, et al. Clinical management of hepatocellular carcinoma. Conclusions of the Barcelona-2000 EASL conference. European Association for the Study of the Liver. *J Hepatol* 2001;35:421-430.
12. Llovet JM, Fuster J, Bruix J, of the Barcelona-Clinic Liver Cancer Group. The Barcelona approach: diagnosis, staging, and treatment of hepatocellular carcinoma. *Liver Transpl* 2004;10:S115-S120.
13. Mazzaferro V, Regalia E, Doci R, et al. Liver transplantation for the treatment of small hepatocellular carcinoma in patients with cirrhosis. *N Eng J Med* 1996;334:697-671.
14. Dusheiko GM, Hobbs KE, Dick R, Burroughs AK. Treatment of small hepatocellular carcinomas. *Lancet* 1992;340:285-288.
15. Castells A, Bruix J, Bru C, et al. Treatment of hepatocellular carcinoma in cirrhotic patients: a cohort study comparing surgical resection and percutaneous ethanol injection. *Hepatology* 1993;18:1121-1126.
16. Livraghi T, Bolondi L, Buscarini L, Cottone M, Mazziotti A, Morabito A, Torzilli G. No treatment, resection and ethanol injection in hepatocellular carcinoma: a retrospective analysis of survival in 391 patients with cirrhosis. *J of Hepatology* 1995;22:522-526.
17. Yamamoto J, Okada S, Shimada K, Okusaka T, Yamasaki S, Ueno H, Kosuge T. Treatment strategy for small hepatocellular carcinoma: comparison of long-term results after percutaneous ethanol injection therapy and surgical resection. *Hepatology* 2001;34:707-713.

18. Llovet JM, Bruix J. Systematic review of randomized trials for unresectable hepatocellular carcinoma: Chemoembolization improves survival. *Hepatology* 2003;37:429-442.
19. Treiber G. Systemic treatment of hepatocellular carcinoma. *Dig Dis* 2001;19:311-323.
20. Aguayo A and Patt YZ. Non surgical treatment of hepatocellular carcinoma. *Semin Oncol* 2001;28:503-513.
21. Leung TW, Johnson PJ. Systemic therapy for hepatocellular carinoma. *Semin Oncol* 2001;28:514-520.
22. Llovet JM, Burroughs A, Bruix J. Hepatocellular Carcinoma. *Lancet* 2003;362:1907-1917.
23. Mathurin P, Rixe O, Bernards B, et al. Review article: overview of medical treatments in unresectable hepatocellular carcinoma- an impossible meta-analysis? *Aliment Pharmacol Ther* 1998;12:111-126.
24. Schwartz JD, Beutler AS. Therapy for unresectable hepatocellular carcinoma: review of the randomized clinical trials-II: systemic and local non-embolization-based therapies in unresectable and advanced hepatocellular carcinoma. *Anticancer Drugs* 2004;15:439-52.
25. Cheng JC, Chuang VP, Cheng SH, et al. Unresectable hepatocellular carcinoma treated with radiotherapy and/or chemo-embolisation. *Int J Cancer* 2001;96:243-252.
26. Cheng JC, Wud JK, Huanga CM, et al. Radiation-induced liver disease after radiotherapy for hepatocellular carcinoma: clinical manifestation and dosimetric description. *Radiother Oncol* 2002;63:41-45.
27. Cheng SH, Lin YM, Chuang VP, Yang PS, Cheng JC, Huang AT and Sung JL. A pilot study of three-dimensional conformal radiotherapy in unresectable hepatocellular carcinoma. *J Gastroenterol Hepatol* 1999;14:1025-1033.
28. Seong J, Park HS, Han KH, et al. Local radiotherapy for unresectable hepatocellular carcinoma patients who failed with arterial chemoembolization. *Int J Radiation Oncology Biol Phys* 2000;47:1331-1335.
29. Lin DY, Lin SM, Liaw YF. Non-surgical treatment of hepatocellular carcinoma. *J Gastroenterol Hepatol* 1997;12:S319-328.
30. Nakakuma K, Tashiro S, Hiraoka T, Ogata K, Ootsuka K. Hepatocellular carcinoma and metastatic cancer detected by iodized oil. *Radiology* 1985;154:15-17.
31. Bhattacharya S, Dhillon AP, Winslet MC, et al. Human liver cells and endothelial cells incorporate iodised oil. *Br J Cancer* 1996;73:877-881.
32. Al-Mufti RA, Pedley RB, Marshall D, Begent RH, Hilson A, Winslet MC, Hobbs KE. In vitro assesment of Lipiodol-targeted radiotherapy for liver and colorectal cancer cell lines. *Br J Cancer* 1999;79:1665-1671.
33. Ho S, Lau WY, Leung TWT and Johnson PJ. Internal radiation therapy for patients with primary or metastatic hepatic cancer. *Cancer* 1998; 83: 1894-1907.
34. Risse JH, Grünwald F, Kersjes W, et al. Intraarterial HCC Therapy with I-131-Lipiodol. *Cancer Biother Radiopharm* 2000;15:65-70.

35. Rindani R, Hugh T, Roche J, Roach PJ, Smith RC. 131I Lipiodol therapy for unresectable hepatocellular carcinoma. *ANZ J Surg* 2002;72:210-214.
36. Raoul JL, Guyader D, Bretagne JF, et al. Prospective randomized trial of chemoembolization versus intraarterial injection of 131I-labeled iodized oil in the treatment of hepatocellular carcinoma. *Hepatology* 1997;26:1156-1161.
37. Bhattacharya S, Novell JR, Dusheiko GM, Hilson AJ, Dick R, Hobbs KE. Epirubicin-Lipiodol chemotherapy versus 131Iodine-Lipiodol radiotherapy in the treatment of unresectable hepatocellular carcinoma. *Cancer* 1995;76:2202-2210.
38. Raoul JL, Guyader D, Bretagne JF, et al. Randomized controlled trial for hepatocellular carcinoma with portal vein thrombosis: intra-arterial iodine-131-iodized oil versus medical support. *J Nucl Med* 1994;35:1782-1787.
39. Lau WY, Leung TW, Ho SK, et al. Adjuvant intra-arterial Lipiodol-iodine-131 for resectable hepatocellular carcinoma: a prospective randomized trial. *Lancet* 1999,353:797-801.
40. Pocock S, White I. Trials stopped too early: too good to be true? *Lancet* 1999;353:943-944.
41. Partensky C Sassolas G, Henry L, Paliard P, Maddern G. Intra-arterial Iodine 131-labeled Lipiodol as adjuvant therapy after curative liver resection for hepatocellular carcinoma. A phase 2 clinical study. *Arch Surg* 2000;135:1298-1300.
42. Raoul JL, Messner M, Boucher E, Bretagne JF, Campion JP, Boudjema K. Preoperative treatment of hepatocellular carcinoma with intra-arterial injection of I131-labelled Lipiodol. *Br J Surgery* 2003;90:1379-1383.
43. Boucher E, Corbinais S, Rolland Y, et al. Adjuvant Intra-arterial Injection of Iodine-131-Labeled lipidol after resection of Hepatocellular Carcinoma. *Hepatology* 2003;38:1237-1241.
44. Brans B, De Winter F, Defreyne L, et al. The anti-tumoural activity of neoadjuvant intra-arterial 131I-Lipiodol treatment for hepatocellular carcinoma: a pilot study. *Cancer Biother Radiopharm* 2001;16:333-338.
45. Wall WJ, Marotta PJ. Surgery and transplantation for hepatocellular cancer. *Liver Transplant* 2000;6:S16-S22.
46. Wang SJ, Lin WY, Chen MN, Hsieh BT, Shen LH, Tsai ZT, Ting G et al. Radiolabelling of Lipiodol with generator-produced 188Re for hepatic tumor therapy. *Appl Radiat Isot* 1996;47:267-271.
47. Wang SJ, Lin WY, Chen MN, Hsieh BT, Shen LH, Tsai ZT, Ting G, et al.. Biodistribution of rhenium-188 Lipiodol infused via the hepatic artery of rats with hepatic tumours. *Eur J Nucl Med* 1996;23:13-17.
48. Jeong JM, Kim YJ, Lee YS, Ko JI, Son M, Lee DS, et al. Lipiodol solution of a lipophilic agent, (188)Re-TDD, for the treatment of liver cancer. *Nucl Med Biol* 2001;28:197-204.
49. Lee YS, Jeong JM, Kim YJ, Chung JW, Park JH, Suh YG, et al. Synthesis of 188 Re-labelled long chain alkyl diaminedithiol for therapy of liver cancer. *Nucl Med Commun* 2002;23:237-242.
50. Qian J, Feng GS and Vogl T. Combined interventional therapies of hepatocellular carcinoma. *World J Gastroenterol* 2003;9:1885-1891.

51. Nishimura Y. Rationale for chemoradiotherapy. *Int J Clin Oncol* 2004;9:414-420.
52. Zhu AX, Willett CG. Chemotherapeutic and biologic agents as radiosensitizers in rectal cancer. *Semin Radiat Oncol* 2003;13:454-468.
53. Boschi A, Uccelli L, Duatti A, Colamussi P, Cittanti C, Filice A, et al. A kit formulation for the preparation of 188Re-Lipiodol: preclinical studies and preliminary therapeutic evaluation in patients with unresectable hepatocellular carcinoma. *Nucl Med Commun* 2004;25:691-699.
54. Abrams RA, Pajak TE, Pajak TF, et al. Survival results among patients with alpha-fetoprotein-positive, unresectable hepatocellular carcinoma: analysis of three sequential treatments of the RTOG and Johns Hopkins oncology center. *Cancer Journal Am Sci* 1998;4:178-184.
55. Brans B, Van Laere K, Gemmel F, et al. Combining iodine-131 Lipiodol therapy with low-dose cisplatin as a radiosensitiser: preliminary results in hepatocellular carcinoma. *Eur J Nucl Med Mol Imaging* 2002;29:928-932.
56. Maeda H. SMANCS and polymer-conjugated macromolecular drugs: advantages in cancer chemotherapy. *Adv Drug Deliv Rev* 2001;46:169-185.
57. Yi SW, Kim YH, Kwon IC, et al. Stable Lipiodolized emulsions for hepatoma targeting and treatment by transcatheter arterial chemoembolisation. *J Control Release* 1998;50:135-143.
58. Yoon CJ, Chung JW, Park JH, et al. Transcatheter arterial chemoembolisation with paclitaxel-Lipiodol solution in rabbit VX liver Tumor. *Radiology* 2003;229:126-131.
59. Pawlik TM and K Keyomarsi. Role of cell cycle in mediating sensitivity to radiotherapy. *Int J Int J Radiat Oncol Biol Phys* 2004;59:928-942.
60. Ulrich-Pur H, Kornek GV, Fiebiger W, Schull B, Raderer M, Scheithauer W. Treatment of advanced hepatocellular carcinoma with bi-weekly high-dose gemcitabine. *Oncology* 2001;60:312-315.
61. Wall JG, Benedetti JK, O'Rourke MA, Natale RB, Macdonald JS. Phase II trial to topotecan in hepatocellular carcinoma: a Southwest Oncology Group study. *Invest New Drugs* 1997;15:257-260.
62. Yang TS, Lin YC, Chen YS, Wang HM, Wang CH. Phase II study of gemcitabine in patients with advanced hepatocellular carcinoma. *Cancer* 2000;89:750-756.

Part 1 Clinical research

1.1. ^{188}Re-HDD/Lipiodol Therapy for Hepatocellular Carcinoma

1.1.1. ^{188}Re-HDD/Lipiodol Therapy for Hepatocellular Carcinoma: A Phase I Clinical Trial

^{188}Re-HDD/Lipiodol Therapy for Hepatocellular Carcinoma: A Phase I Clinical Trial

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ABSTRACT

The present study aimed to investigate the pharmacokinetics, organ dosimetry and toxicity following the intra-arterial administration of ^{188}Re-4-hexadecyl-1-2,9,9-tetramethyl-4,7-diaza-1,10-decanethiol/Lipiodol (^{188}Re-HDD/Lipiodol) for palliative treatment of hepatocellular carcinoma (HCC). A secondary objective was to document the response.
Methods: A mean activity of 3.60 GBq ^{188}Re-HDD/Lipiodol (range: 1.86-4.14 GBq) was administered to 11 patients (16 treatment sessions) via a transfemoral catheter. The pharmacokinetic and dosimetric data were collected by means of venous blood samples, urine collections and 4 to 5 gamma scintigraphies over 76 hours. Absorbed doses to the various organs were calculated according to the Medical Internal Radiation Dose (MIRD) formalism, using the MIRDDOSE© 3.1 software. The toxicity was assessed until 6 weeks post-administration by means of the CTC-scale. The response was evaluated on MRI and by monitoring of the tumor marker.
Results: A fast blood clearance of the injected activity was observed with a calculated effective half-life of 7.6 (± 2.2 SD) hours in blood. The predominant elimination of the activity was through urinary excretion with a mean renal clearance of 44.1 (± 11.7 SD) % of the injected activity within the 76 hours after administration. Fecal elimination was negligible. The calculated whole body effective half-life was 14.3 (± 0.9 SD) hours. The absorbed dose to the liver tissue, the lungs, the kidneys and the thyroid was 4.5 ± 1.9 Gy, 4.1 ± 1.2 Gy, 0.9 ± 0.7 Gy and 0.3 ± 0.1 Gy, respectively. Treatment was well tolerated, except in two patients. One Child B patient experienced a worsening of his liver dysfunction (hyperbilirubinaemia) and another patient experienced dyspnea and coughing. Response assessment on MRI showed one case of partial response,

disease stabilization in 11 treatments and progressive disease in 1 treatment. In 5 out of 8 treatment sessions with an initially elevated alpha-fetoprotein a reduction (range 19-90%) was observed 6 weeks later.
Conclusion: After the intra-arterial administration of 3.60 GBq ^{188}Re-HDD/Lipiodol, a fast clearance of the activity appearing in the blood is observed and the predominant elimination is through urinary excretion. The tolerance as well as the preliminary response rates of the present phase I study are encouraging.

INTRODUCTION

Hepatocellular Carcinoma or HCC is the most prevalent primary liver cancer. It constitutes the third cause of cancer-related deaths, responsible for more than 500 000 deaths worldwide annually (*1*). Although the overall incidence of HCC in the West is low compared to South East Asia, Sub-Saharan Africa and Japan, its incidence is rising (*2*,*3*). Due to the underlying carcinogenic risk factors such as chronic viral hepatitis and alcohol intake, the vast majority of patients presenting with HCC, have cirrhosis, a condition limiting the therapeutic possibilities. Surgery, either by means of hepatectomy or liver transplantation, is the mainstay of curative treatment. Overall, if the degree of liver dysfunction and the tumor load is taken into consideration, the vast majority of patients is not eligible for surgery (*4*).

Percutaneous alcohol injection, (chemo-)embolization and radiofrequent ablation are among the most common treatment modalities (*5*). Survival advantages were identified with chemo-embolization in well selected patients (*6*). Other treatment modalities producing response rates exceeding 20% are the use of Lipiodol mixed with chemotherapeutic agents or radiolabeled with Iodine-131 (^{131}I) without subsequent embolization (*7*). Encouraging results have been obtained using ^{131}I-Lipiodol, but the use of ^{131}I has hampered its routine clinical implementation (*8,9*). ^{131}I has a physical half-life of 8 days and emits a high energetic gamma-ray (364 keV, with an abundancy of 82%) necessitating delayed hospitalization for radioprotection purposes. Although ^{131}I-Lipiodol therapy is generally well tolerated, a dose escalation study was not conducted so far as the administration of activities exceeding 2.22 GBq is restricted by the above mentioned practical issues. Rhenium-188 (^{188}Re) has favorable characteristics for radionuclide therapy and considering the limited success of ^{131}I-Lipiodol for treatment of relatively large tumors, the switch towards a radionuclide with a higher energy of the beta-emission (2120 keV and 1960 keV for ^{188}Re versus 606 keV for ^{131}I) might yield improved response rates (*10*). ^{188}Re emits a gamma ray of 155 keV at an abundancy of 15%, allowing gamma-camera imaging and it has a relatively short physical half-life of 17 hours, limiting radiation protection problems. Additionally, the radionuclide is eluted from a ^{188}W/^{188}Re- generator, which has a long useful shelf-life of several months and provides a good yield of carrier-free ^{188}Re routinely (*11*).

During the past ten years, promising pre-clinical results using ^{188}Re labeled Lipiodol for treatment of HCC have been reported (*12-14*). Only recently, however, were the first clinical results using ^{188}Re-4-hexadecyl-1-2,9,9-tetramethyl-4,7-diaza-1,10-decanethiol/Lipiodol (^{188}Re-HDD/Lipiodol) reported by Sundram and coworkers (*15,16*). The aim of the present study was to assess the biodistribution and feasibility of a locoregional administration of 3.7 GBq of ^{188}Re-HDD/Lipiodol in palliative HCC-patients.

MATERIALS AND METHODS

Synthesis and Quality Control of the Radio-conjugate

$^{188}W/^{188}Re$-generators were purchased from the Oak Ridge National Laboratory, (Tennessee, USA) and the IRE (Institut des Radio-Eléments, Fleurus, Belgium). Lyophilised kits containing a HDD-chelator (4-hexadecyl-1-2,9,9-tetramethyl-4,7-diaza-1,10-decanethiol) were provided by the Seoul National University Hospital and ^{188}Re-HDD/Lipiodol was synthesized as described earlier by Jeong et al.(*13*). Briefly, the concentrated eluate (6 mL) from a commercially available $^{188}W/^{188}Re$ generator containing 11.1 GBq, was heated with the HDD/$SnCl_2$ kit at 100°C for 1 hour to produce ^{188}Re-HDD complex (*17*). 3 mL of Lipiodol was added and mixed on a vortex to extract the ^{188}Re-HDD into the Lipiodol. After centrifugation at 4000g the ^{188}Re-HDD/Lipiodol fraction was separated. Finally, the ^{188}Re-HDD/Lipiodol layer was washed with a 0.9% NaCl solution. Quality control was performed according to the method described by Jeong et al. by ITLC (ITLC-SG plates, Gelman Sciences, mobile phase: 0.9% NaCl and Acetone) (*14*). Radiochemical purity was in all cases higher than 95%. The total radiochemical yield was 53% ± 4.5 %.

Patient Selection

The diagnosis of HCC had to be established by means of CT-scan, Magnetic Resonance Imaging (MRI) or arteriography, eventually in conjunction with an elevated alpha-fetoprotein (AFP) level or biopsy. Exclusion criteria were: eligibility for liver resection or transplantation, pregnancy and breast feeding, age <18 years, Child C status according to the modified Child-Pugh score, Okuda III disease stage, poor general condition (Karnofsky <70%), white blood cell count <1500/µl and potentially toxic anticancer treatment in the preceding 3 months. Additional contra-indications for the arteriography and isolation procedure were the following: serum creatinine level >2 mg/dl, prothrombin time <50 % of the normal value, platelet count <50 000/µl, inability of self-care, encephalopathy and incontinence. The study was approved by the institutional ethic's committee and an informed written consent was obtained from all patients.

Administration

Under local anesthesia, a 5 French catheter was inserted transfemorally and introduced into the proper hepatic artery. After obtaining a diagnostic hepatic arteriogram, approximately 4 ml of ^{188}Re-HDD/Lipiodol was injected slowly into the proper hepatic artery under fluoroscopic control. Whenever aberrant arterial supply was present, the radio-conjugate was injected selectively into the right and left hepatic artery separately. The volume of 4 ml was divided over the different hepatic arteries proportional to the volume of liver parenchyma supplied by the respective artery. To reduce uptake of free perrhenate in the thyroid or gastric mucosa, patients received 0.5 gram sodium perchlorate prior ^{188}Re-HDD/Lipiodol administration followed by 1 gram daily until discharge.

Pharmacokinetic and Dosimetric Study

Following ^{188}Re-HDD/Lipiodol administration patients were hospitalized in a dedicated radionuclide therapy room for 4 days. In all patients, whole body scintigraphies were acquired at 1.7 (SD 1.2) , 20.0 (SD 1.9), 27.6 (SD 1.9), 51.7 (SD 1.8) and 75.4 (SD 1.8) hours post-administration using a triple headed gamma camera (IRIX®, Philips, Eindhoven, The Netherlands) equipped with medium energy parallel hole collimators. The imaging window was set at 155 keV (20%). Scan speed varied from 30 to 10 cm/minute depending upon the time elapsed since administration. For quantification purposes a syringe containing a known activity of ^{188}Re was included in the whole body scan.

During the hospitalization period, patients collected their urine. Prior to each whole body scan, urinary volumes were recorded and samples taken. On these occasions, venous blood samples were also taken. Blood and urine samples were analyzed in a NaI(Tl) 3"x3"gamma well counter calibrated for ^{188}Re (Cobra II, Perkin Elmer, USA). Time-activity curves were generated for blood and urinary activity and fitted mono-exponentially (SPSS software version 10.0, Chicago, USA).

For data analysis, whole-body images were transferred to a HERMES® system (Nuclear Diagnostics, Sweden). Regions of interests (ROI) were drawn around the syringe, the total body, the liver (including tumor), the lungs and a background region on the first scan. ROIs were mirrored to the posterior image and copied to subsequent scans. The background corrected geometric mean of the total counts in the ROIs was used to calculate the total amount of activity in the total body, the liver and the lungs, using the known activity in the syringe. Experimental factors were determined on an anthropomorphic phantom (Alderson Heart/Thorax SPECT phantom) for the conversion of the syringe activity into organ activity. The overall uncertainty using this methodology for the activity calculation was less than 18%.

Mono-exponential time activity curves were generated for the total body, the liver and lungs using SPSS 10.0 software. Source organ residence times were determined from integration of the time activity curves. Absorbed doses to the various organs were calculated according to the Medical Internal Radiation Dose (MIRD) formalism, using the MIRDDOSE© 3.1 (Oak Ridge Associated Universities, USA) software package (*18*). Using the time-activity curves of the blood samples, red marrow absorbed dose was calculated according to the methodology described by Sgouros et al. (*19*).

The patient's dose rate was regularly measured at one meter distance of the liver region using a survey meter.

Toxicity and Response Assessment

Laboratory testing of red and white blood cells and platelets, liver function, renal function and serum alpha-fetoprotein were performed short before treatment, on the third day and 2 and 6 weeks post-injection. Clinical evaluation of toxicity was performed daily during hospitalization and 2 and 6 weeks later. Toxicity was scored by means of the Common Toxicity Criteria (Cancer Therapy Evaluation Program. Common Toxicity Criteria, Version 2.0. Division of Cancer Treatment and Diagnosis, National Cancer Institute, National Institutes of Health, Department of Health and Human Services, March 1998).

Laboratory findings were compared by means of Wilcoxon statistical testing and the significance level was set at $P < 0.05$ (SPSS version 10.0). Radiological response was assessed by means of the RECIST response criteria (response evaluation criteria in solid tumors) on MRI images acquired 6 weeks following treatment (*20*). Patients without evidence of progressive disease were eligible for repetitive treatment sessions with a 12 week interval.

RESULTS

Eleven patients underwent 16 treatment sessions. The mean administered activity per session was 3.60 GBq (range: 1.86-4.14 GBq). Eight patients were treated once, 1 patient twice and 2 patients thrice. Patient characteristics are summarized in Table 1.

TABLE 1: Patient characteristics
Risk factors: chronic hepatitis B infection (HBV), chronic hepatitis C infection (HCV), alcohol (ALC), hemochromatosis (HC), none (NO). Modified Child-Pugh score: not applicable because of absence of cirrhosis (NA). Previous treatment: none (NO), ^{131}I- Lipiodol (I131), trans-arterial chemo-embolization (TACE), radiofrequent ablation (RFA).

Nr	**Age** (years)	**Sex**	**Risk factor**	**Modified Child-Pugh score**	**Okuda score**	**Number of sessions**	**Previous treatment** (interval in weeks)
1	71	M	ALC	B9	II	1	NO
2	71	M	HCV, ALC	B9	II	1	I131 (16 w)
3	71	F	HCV	A5	I	3 (a, b, c)	I131 (14 w)
4	69	M	ALC	A5	I	1	I131(16 w)
5	58	F	HCV	A5	I	1	NO
6	72	M	HCV	A5	I	2 (a, b)	RFA (52 w)
7	76	M	HC	A5	I	3 (a, b, c)	NO
8	59	M	ALC	A6	II	1	RFA (52 w)
9	53	F	NO	NA	I	1	TACE, I131 (14 w)
10	58	M	ALC	A6	II	1	I131 (18 w)
11	77	M	HCV	A5	I	1	I131 (16 w)

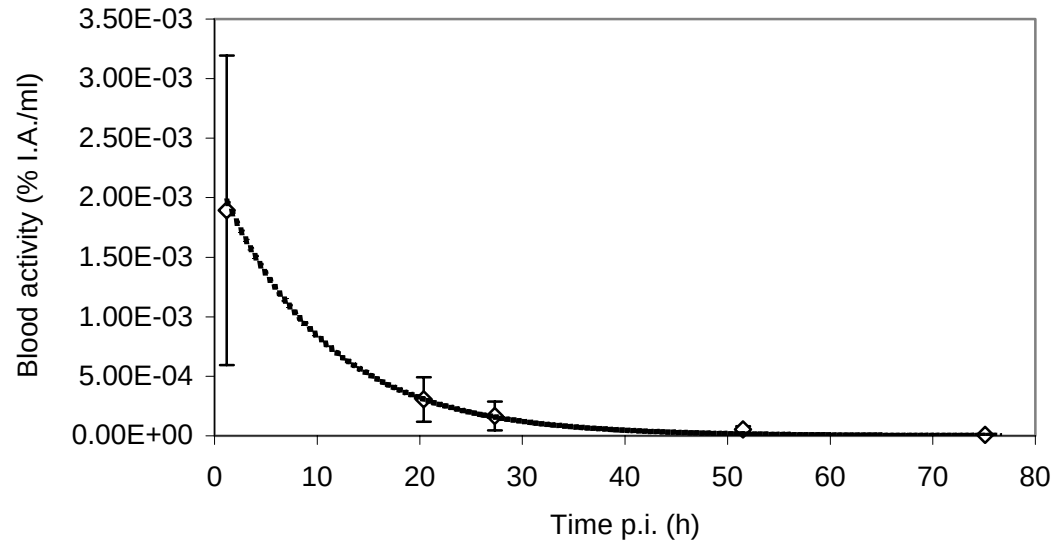
Pharmacokinetic Data and Organ Dosimetry

In 7 treatment sessions a minimum of 4 blood samples were taken within 76 hours of administration. A fast blood clearance of the injected activity was observed, with a calculated mean effective half-life of 7.6 (± 2.2 SD) hours in blood. The estimated blood activity at time 0 was 77.2 (± 64.4 SD) kBq/ml.
Urinary excretion was assessable during hospitalization in all treatments. A mean of 44.1 (± 11.7 SD)% of the administered activity was excreted within 76 hours following administration. The largest fraction was excreted within the first 21 hours (25.9 ± 6.3% SD). ITLC analysis showed that the activity in the urinary samples was perrhenate. Blood activity levels and cumulative urinary

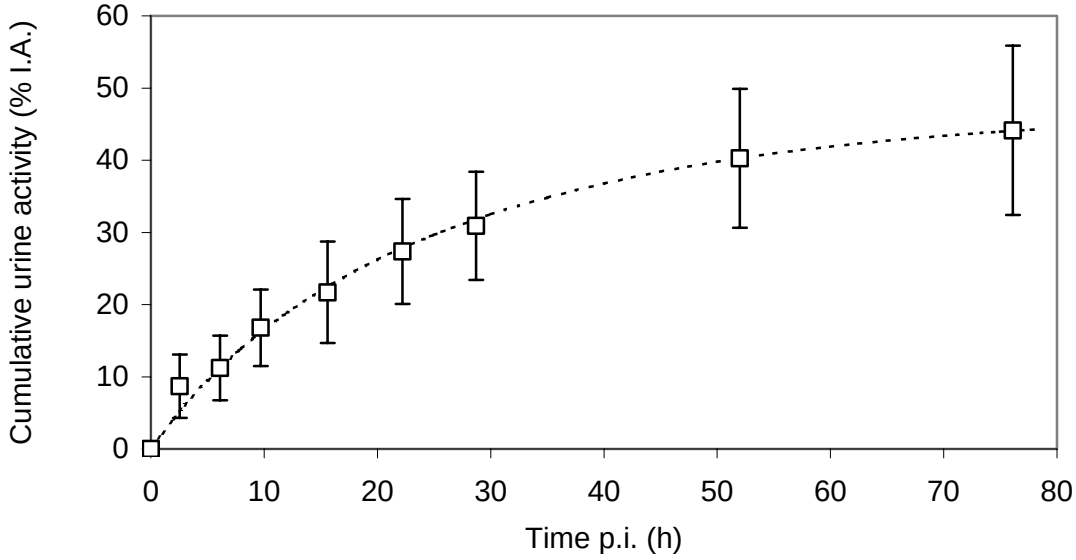
excretion of the injected activity are plotted as a function of time in Figure 1. Based on the urinary excretion levels, a mean whole body effective half live of 14.3 (± 0.9 SD) hours was calculated using a mono-exponential fitting.
Feces was collected on three occasions; fecal elimination proved negligible (mean 0.9 ± 0.2% SD of the administered activity). Scintigraphies showed activity in the small bowel and colon, which was faint on 4 occasions and significant on one occasion.

FIGURE 1: Blood activity levels in percent of the injected activity per milliliter blood as a function of time post injection (A). Cumulative urinary excretion of activity in percent of the injected activity plotted as a function of time post-injection (B).

A



B

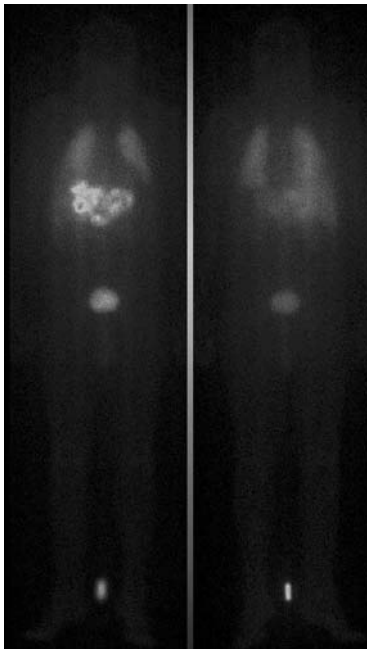


The absorbed doses to the organs were calculated in all treatment sessions except one (session number 7c) because of technical problems (Table 2). The MIRDOSE red marrow absorbed dose (0.30 ± 0.08 Gy) is in good agreement with the red marrow dose calculation based on the methodology of Sgouros (0.44 ± 0.21 Gy). No thyroid activity was seen on the scintigraphies (Figure 2). The mean effective dose estimation was 1.0 (± 0.2 SD) Sv.
The dose rate at one meter dropped below 20μSv/h within the first 24 hours following administration and did not exceed 5 μSv/h on day 2.

TABLE 2: The absorbed doses (Gy) for the various normal organs after treatment with mean 3.34 (± 0.93) GBq ^{188}Re-HDD/Lipiodol. LLI (lower large intestine), ULI (upper large intestine).

Target Organ	**Absorbed Dose (Gy)**		
	Mean	**SD**	**Range**
Liver	4.49	1.87	1.84 – 7.25
Lungs	4.07	1.18	1.38 – 5.96
ULI Wall	0.89	0.80	0.21 – 3.91
Kidneys	0.89	0.71	0.24 – 2.58
Stomach	0.30	0.08	0.14 – 0.41
LLI Wall	0.30	0.08	0.14 – 0.42
Thyroid	0.30	0.07	0.13 – 0.40
Red Marrow	0.30	0.08	0.14 – 0.43

FIGURE 2: Whole body scan (right: anterior image, left: posterior image) of patient number 4 performed 30 hours after administration of 4.12 GBq ^{188}Re-HDD/Lipiodol. Significant uptake is seen in the multifocal hepatocellular carcinoma and in the bladder. Faint uptake is observed in the lungs and no uptake in the thyroid nor gastric mucosa.



Toxicity

In Table 3 the adverse events are scored according to the Common Toxicity Criteria version 2.0. Only the events reflecting a worsening compared to the baseline values are tabulated. Patient number 1 received a "special request" liver transplantation after 5 weeks, excluding him from subsequent toxicity assessment and patient number 11 did not attend follow-up consultations due to his limited general condition.

Hematology. No significant changes in blood counts were observed on week 2. On week 6 there was a borderline significant decrease in white blood cells and platelets (*P*-values respectively 0.048 and 0.045). Subanalysis pointed out that there were no significant changes at all in the blood counts if patient number 6 was not taken into consideration. This patient developed a transient grade 2 leukopenia and a grade 3 thrombocytopenia. Values for white blood cells and platelets before therapy and on week 6 are depicted in Figure 3 A and B.

Liver Function. No clinical liver toxicity was encountered, except in patient number 1. This Child B patient had a pre-existing elevated bilirubin level and a history of spontaneous liver decompensation. He developed a fatigue grade 2 and an aggravation of his icterus. Overall, no significant increases were seen in the bilirubin, AST nor ALT levels at week 2 and 6 (Figure 3 C and D). There was no significant change in the modified Child-Pugh score 6 weeks following treatment.

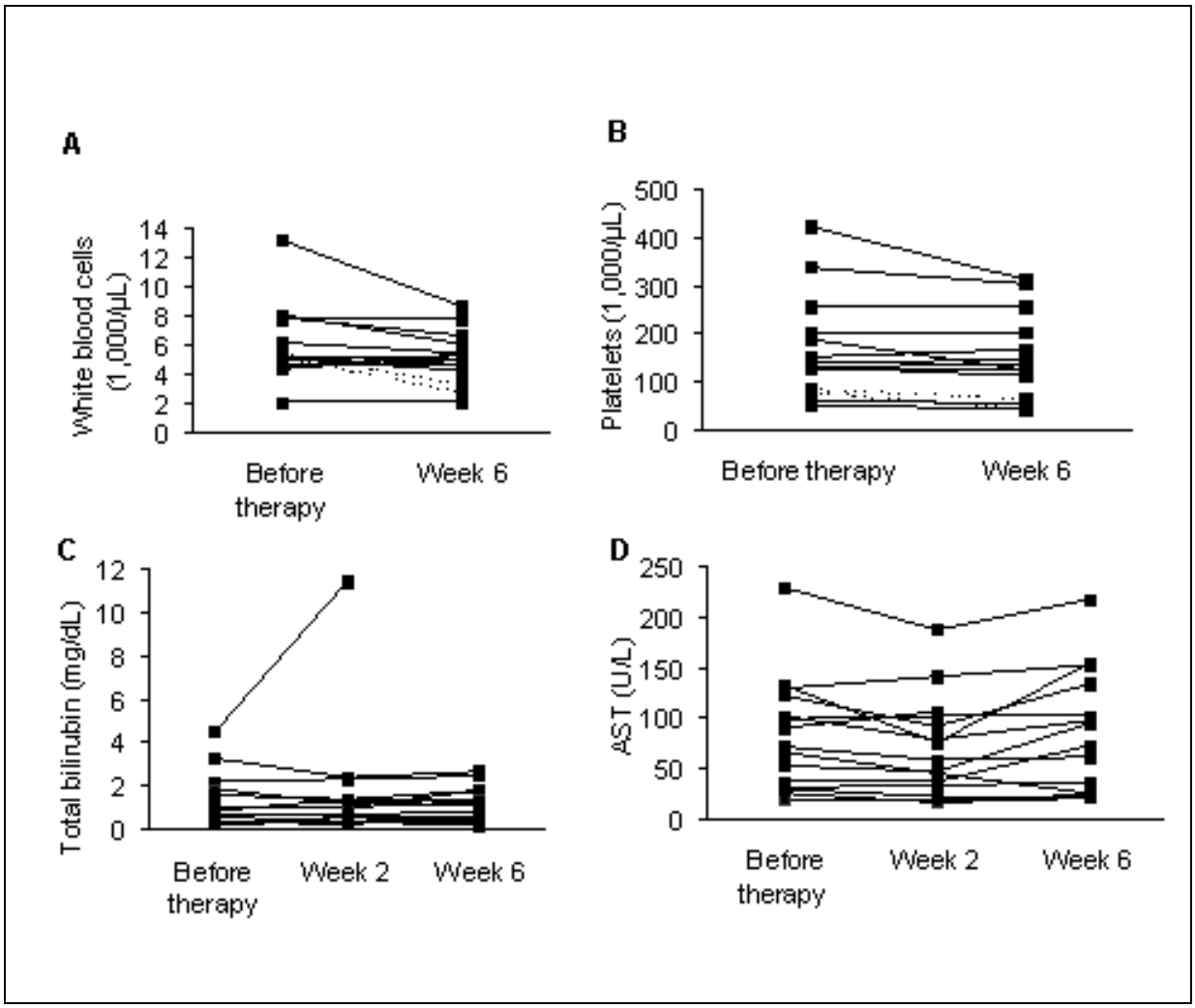
Lung Function. No acute pulmonary symptoms were reported during the hospitalization. Patient number 6 developed a cough after his first treatment session. Lung function testing did not reveal any contra-indications for further treatment with ^{188}Re-HDD/Lipiodol. However, after the second session dyspnea and cough reoccurred and subsequent lung function testing showed a deterioration of the diffusion capacity (DLCO 43% of the predicted value) and bilateral fibrotic changes were observed on high resolution CT-scan of the lungs. After oral steroid treatment, spirometric changes resolved and symptomatic relief was rapidly achieved. Since the patient's compliance to the steroid treatment was unsatisfactory in the late follow-up, symptoms reoccurred several months later.

Other. No significant changes were observed in the creatinine levels 2 and 6 weeks after the procedure. Besides the toxicity possibly related to the investigational agent, some adverse events were due to the catheterization: a small groin hematoma in 3 patients and a grade 1 allergic reaction in a patient, with a history of a similar reaction following ^{131}I-Lipiodol treatment.

TABLE 3: Scoring of adverse events according to the Common Toxicity Criteria version 2.0.

Session	Adverse event	Grade	Attribution to investigational agent
1	fatigue (day 2)	2	possibly
	increase in bilirubin (week 2)	3	possibly
	increase in AST (day 2-week 2)	2	possibly
3a	colitis (week 2)	2	unlikely
4	diarrhea (day 2)	1	unrelated
	tumor pain (week 2)	1	possibly
6a	infection with fever (week 2)	2	unlikely
	leukopenia (week 6)	2	probably
	thrombocytopenia (week 6)	2	probably
	cough (week 6)	2	possibly
6b	leukopenia (week 6)	1	probably
	thrombocytopenia (week 6)	3	probably
	cough, dyspnea, pulmonary fibrosis (week 6)	2	possibly
	reduced carbon monoxide diffusion capacity	3	possibly
7c	tumor pain (week 2)	1	possibly
8	fatigue (week 2)	2	possibly
9	fatigue (week 2)	2	probably
	increase in AST (week 2)	2	probably
10	infection with fever (day 2-7)	1	unrelated

FIGURE 3: White blood cell counts before therapy and at week 6 (A), platelet counts before therapy and at week 6 (B). The dotted lines represent sessions number 6a and b. Bilirubin levels before therapy and at week 2 (C), AST levels before therapy and at week 2 (D).

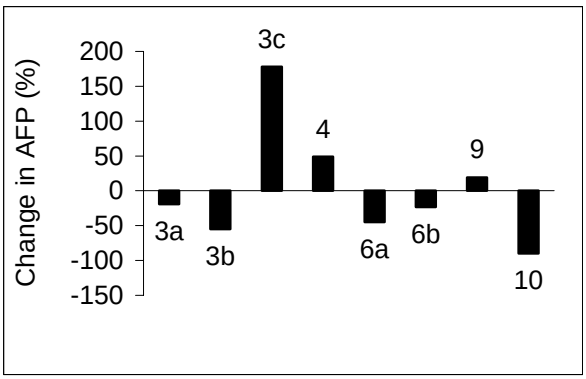


Response

Response on MRI. Patient number 10 suffered from a rapidly progressive multifocal tumor with grade 4 anaplastic disease on pathology. In this patient a partial response was obtained with a reduction of the sum of largest diameters by 41%. Patient number 3 suffered from progressive disease after her third treatment with ^{188}Re-HDD/Lipiodol. Patients number 1 and 11 dropped out for response assessment 6 weeks after treatment and patient number 5 was not assessable because imaging was performed on different scanners. The remaining patients had stable disease.

Response on AFP. Figure 4 depicts the change in serum AFP of baseline values at week 6. In 8 treatment sessions AFP levels were elevated prior to treatment. In 5 out of these treatment sessions a reduction in tumor marker (range 19-90%) was observed 6 weeks later.

FIGURE 4: Change in serum AFP of baseline values at week 6 in 8 treatment sessions with an initially elevated tumor marker level.



DISCUSSION

Taking into account the effective half-life, beta-energy and dose rate, the same effect on the tumor may be expected by ^{188}Re-HDD/Lipiodol and ^{131}I-Lipiodol when the administered ^{188}Re-activity is 60% higher than the ^{131}I-activity (*21*). This calculation is based upon iso-effect curves for different dose rates used in brachytherapy (*22*). Therefore we aimed in the present study at an administration of 3.7 GBq of ^{188}Re-HDD/Lipiodol because before initiation of this study, a fixed activity of 2.2 GBq ^{131}I-Lipiodol was usually applied at our institution. A non-selective administration in the hepatic arterial vessels was preferred because development of metachronous HCC in the non-treated segments is described in case the administration of the activity is restricted to the affected segments or lobe (*23*).
Concerning the labeling procedure, no major technical problems were encountered in 15 out of 16 syntheses. The hospital stay in isolation for up to 7 days in certain European countries in order to comply with radioprotective guidelines, is among the most important constraints of the use of ^{131}I-Lipiodol. The patient's dose rate measured at 1 meter dropped below the local limit of 20 µSv/h within 24 hours following administration of ^{188}Re-HDD/Lipiodol, allowing a significant shorter hospitalization. The shorter stay in the shielded therapy room and the use of on-site ^{188}W/^{188}Re-generator improved the flexibility in treatment planning.

Since ^{188}Re is differently attached to Lipiodol (coordinative binding with a chelator) compared to ^{131}I-Lipiodol (covalent binding), differences in metabolism can be expected. We observed a significant accumulation of the radio-conjugate in the liver and lungs and, to a much smaller amount, uptake in the gastrointestinal tract and kidneys. The significant lung uptake is in agreement with the initial clinical experiences with ^{188}Re-HDD/Lipiodol reported by Sundram et al. and with the previous studies on the biodistribution of ^{131}I-Lipiodol *(15,16,24-26).*
We calculated a biologic half-life in the lungs for ^{188}Re of 8.7 (± 4.9 SD) days and this result is in line with our earlier report on the biologic half-life in the lungs following ^{131}I-Lipiodol therapy of 10.3 (± 5.2 SD) days (*27*). The relatively

long biologic lung half-life in both cases suggests that the activity in the lung is present under a stable formulation from which diffusion to blood is minimal.
In the present study a considerable number of patients showed faint uptake in other organs such as the kidneys and parts of the gastro-intestinal tract. The data available in the literature concerning the elimination of ^{131}I-Lipiodol are scarce but all investigators agree on the predominant urinary excretion and negligible elimination of ^{131}I in stool. The largest observation comprising measurements in 10 patients suffering from HCC yielded a median of 42% of the administered ^{131}I excreted urinary within the first 8 days (*24*). Nakajo et al. obtained comparable results with a daily excretion from 4.3 up to 7.1% (*25*). In a small biodistribution study conducted by Madsen et al. in 4 patients, a lower daily excretion of ^{131}I (1-3%) was observed (*26*). In the experiences with ^{188}Re-HDD/Lipiodol reported by Sundram et al. a faint visualization of the kidneys was described and they concluded that renal elimination was negligible. However, the urinary excretion was not quantified (*16*). According to our measurements a mean of 44.1% of the administered ^{188}Re was excreted in the urine within 76 hours. If the shorter physical half-life of ^{188}Re is taken into account, this value compares favorably with the observations of Raoul et al. and Nakajo et al. (*24,25*).

No visualization of the gastro-intestinal tract was described in the biodistribution studies using ^{131}I-Lipiodol nor in the initial reports on ^{188}Re-HDD/Lipiodol (*15,16,24-26*). However, in our experience in 5 out of 16 administrations transient uptake in the small or large bowel was observed, which was very faint in all patients but one. In the animal study conducted by Lee et al. intestinal uptake was observed following intravenous administration of ^{188}Re labeled long chain alkyl diaminedithiol derivatives in mice and the intestinal activity was thought to be result of bile excretion (*14*). In our study the uptake pattern and its transient character suggested biliary excretion rather than gastro-intestinal wall uptake.

Only few data are available on the blood activities following ^{131}I-Lipiodol administration. Measurements performed by Nakajo et al. and Madsen et al. pointed out that less than 0.9% of the administered activity was retrieved in the total blood volume with a peak in blood activity after 3 days (*25,26*). This was in contrast with the activities measured in the present study, indicating an initially significant higher blood activity about 10% of the administered activity ^{188}Re-HDD/Lipiodol, with a rapid decrease over the first 3 days. Sundram et al. performed activity measurements in 5 patients short after the administration of a "scout dose" of 200 MBq ^{188}Re-HDD/Lipiodol. The obtained activities were considered negligible but the biodistribution data are difficult to compare since most studies vary in the selectivity of administration of the radio-conjugate. (*15,16*).

Absorbed doses to the liver, lungs and thyroid calculated for 1.90 $\pm$ 0.20 GBq ^{131}I-Lipiodol therapy, are significantly higher (7.8 $\pm$ 1.8 Gy, 6.8 $\pm$ 2.9 Gy and 7.2 $\pm$ 2.2 respectively) compared to the absorbed doses in this ^{188}Re-HDD/Lipiodol study population (*27*). The mean effective dose of the ^{188}Re-HDD/Lipiodol

patients (1.0 ± 0.2 Sv) was significantly lower compared to ^{131}I-Lipiodol (2.0 ± 0.6 Sv).

The initial clinical experiences using ^{188}Re-HDD/Lipiodol have been described by Sundram and coworkers. In this study, about 35 out of 70 patients did not experience any adverse event. The most frequent adverse events consisted of mild anorexia, right hypochondrial discomfort and low grade fever (*16*). We had a similar observation with the occurrence of in general only mild toxicity in 9 out of 16 treatment sessions. Patient number 1 suffered from a significant worsening of his liver dysfunction. Although this patient had a history of frequent liver decompensation and his tumor marker was steeply increasing at week 2, we recommend that additional data are acquired to confirm the safety of ^{188}Re-HDD/Lipiodol therapy in Child B liver cirrhosis patients. No cases of liver failure were reported by Sundram and coworkers (*15,16*). However, in that trial the activity was administered as close to the tumor feeding artery as possible whereas the present study aimed to treat the whole liver and therefore the radio-conjugate was injected non-selectively. Sundram et al. reported 2 cases of pleural effusion and these were attributed to radiation induced pneumonitis (*16*). In our study patient number 6 developed grade 3 lung toxicity 6 weeks after his second treatment. The absorbed lung doses of sessions 6 a en b were estimated to be respectively 4.6 Gy and 5.8 Gy, hence too low to explain this evolution. The timing of the occurrence of his symptoms and the associated bone marrow depression are suggestive for an increased sensitivity to ionizing radiation in this patient. No bone marrow depression was observed in the other patients, including the population described by Sundram et al. (*16*). Although is was not clear if the occurrence of lung fibrosis in this patient was related to the ^{188}Re-HDD/Lipiodol therapy, we advocate that in the future particular attention is paid to patients developing pulmonary complaints. If radiation induced pneumonitis is detected, adequate prevention of fibrosis by means of steroid treatment is recommended.

Besides the excellent tolerance of the treatment in the majority of patients, the preliminary response rates in this study were encouraging, since 6 out of 11 patients had clear evidence of progressive disease at inclusion.
Future research should attempt to elucidate various issues concerning the maximum tolerated activity and the long term effects of the treatment on the lung tissue. Future work should also focus on the optimization of the administration protocol in terms of selectivity and the use of a fixed activity in stead of estimating the optimal activity following the administration of a 'scout-dose'. In addition, efforts should be made in these studies to perform SPECT-tumor dosimetry. As more data on the recommended activities and treatment intervals become available, the cost effectiveness of this treatment modality should be investigated.

CONCLUSION

A mean activity of 3.60 GBq ^{188}Re-HDD/Lipiodol was administered to 11 patients in 16 treatment sessions. A fast blood clearance of the injected activity was observed with a calculated effective half-life of 7.6 (± 2.2 SD) hours in blood. The predominant elimination of the activity was through urinary excretion with a mean renal clearance of 44.1 (± 11.7) % of the injected activity within the 76 hours following administration. The calculated whole body effective half-life was 14.3 (± 0.9 SD) hours. Treatment was well tolerated, except in two patients. The results of the present phase I study are encouraging and according to the dosimetric estimations a further dose escalation study is warranted.

REFERENCES

1. Parkin DM, Bray F, Ferlay J, Pisani P. Estimating the world cancer burden: globocan 2000. *Int J Cancer* 2001;94:153-156.
2. El-Serag HB, Masson AC. Rising incidence of hepatocellular carcinoma in the United States. *N Eng J Med* 1999;340:745-750.
3. El-Serag HB, Davila JA, Petersen NJ, McGlynn KA. The continuing increase in the incidence of hepatocellular carcinoma in the United States: an update. *Ann Intern Med* 2003;139:817-823.
4. Schafer DF, Sorrell MF. Hepatocellular carcinoma. *Lancet* 1999;353:1253-1257.
5. Giovannini M, Elias D, Monges G, Raoul JL, Rougier P. Hepatocellular carcinoma. *Br J Cancer* 2001;84:74-77.
6. Llovet JM, Bruix J. Systematic review of randomized trials for unresectable hepatocellular carcinoma: chemoembolization improves survival. *Hepatology* 2003;37:429-442.
7. Llovet JM, Burroughs A, Bruix J. Hepatocellular carcinoma. *Lancet* 2003; 362:1907-1917.
8. Raoul JL, Guyader D, Bretagne JF, et al. Prospective randomized trial of chemoembolization versus intra-arterial injection of 131-I-iodized oil in the treatment of hepatocellular carcinoma. *Hepatology* 1997;26:1156-1161.
9. Leung WT, Lau WY, Ho S, et al. Selective internal radiation therapy with intra-arterial iodine-131-Lipiodol in inoperable hepatocellular carcinoma. *J Nucl Med* 1994;35:1313-1318.
10. Risse JH, Grünwald F, Kersjes W, et al. Intra arterial HCC therapy with 131I-Lipiodol. *Cancer Biother Radiopharm* 2000;15:65-70.
11. Knapp FF. Rhenium -188- A generator- derived radioisotope for cancer therapy. *Cancer Biother Radiopharm* 1998;13:337-349.
12. Wang SJ, Lin WY, Chen MN, et al. Biodistribution of rhenium-188 Lipiodol infused via the hepatic artery of rats with hepatic tumours. *Eur J Nucl Med* 1996;23:13-17.
13. Jeong JM, Kim YJ, Lee YS, et al. Lipiodol solution of a lipophilic agent, 188-Re-TDD, for the treatment of liver cancer. *Nucl Med Biol* 2001;28:197-204.
14. Lee YS, Jeong JM, Kim YJ, et al. Synthesis of 188Re-labelled long chain alkyl diaminedithiol for therapy of liver cancer. *Nucl Med Commun* 2002;23:237-242.
15. Sundram FX, Jeong JM, Zanzonico P, et al. Trans-arterial Rhenium-188 Lipiodol in the treatment of inoperable hepatocellular carcinoma. An IAEA sponsored multi-centre phase 1 study. *World J Nucl Med* 2002;1:5-11.
16. Sundram F, Chau TC, Onkhuudai P, et al. Preliminary results of transarterial rhenium-188 HDD Lipiodol in the treatment of inoperable primary hepatocellular carcinoma. *Eur J Nucl Med Mol Imaging* 2004;31:250-257.
17. Guhlke S, Beets AL, Oetjen K, et al. Simple new method for effective concentration of Re-188 solutions from alumina-based W-188-Re-188 generator. *J Nucl Med* 2000;4:1271-1278.
18. Stabin MG. MIRDOSE: personal computer software for internal dose assessment in nuclear medicine. *J Nucl Med* 1996;37:538-546.

19. Sgouros G. Bone marrow dosimetry for radioimmunotherapy: theoretical considerations. *J Nucl Med* 1993;34:689-694.
20. Therasse P, Arbuck SG, Eisenhauer EA, et al. New guidelines to evaluate the response to treatment in solid tumors. *J Natl Cancer Inst* 2000;92:205-216.
21. De Ruyck K, Lambert B, Bacher K, et al. Biological dosimetry of 188Re-HDD/Lipiodol versus 131I-Lipiodol therapy in patients with hepatocellular carcinoma. *J Nucl Med* 2004;45:612-618.
22. Steel GG. The dose-rate effect: brachytherapy. In: Steel GG, ed. Basic Clinical Radiobiology. London, UK: Edward Arnold Publishers; 1993,120-129.
23. Brans B, Bacher K, Vandevyver V, et al. Intra-arterial radionuclide therapy for liver tumours: effect of selectivity of catheterization and 131I-Lipiodol delivery on tumour uptake and response. *Nucl Med Commun* 2003;24:391-396.
24. Raoul JL, Bourguet P, Bretagne JF, et al. Hepatic artery injection of I-131-labeled Lipiodol. Biodistribution study results in patients with hepatocellular carcinoma and liver metastases. *Radiology* 1988;168:541-545.
25. Nakajo M, Kobayashi H, Shimabukuro K, et al. Biodistribution and in vivo kinetics of iodine- 131 Lipiodol infused via the hepatic artery of patients with hepatic cancer. *J Nucl Med* 1988;29:1066-1077.
26. Madsen MT, Park CH, Thakur ML. Dosimetry of iodine-131 ethiodol in the treatment of hepatoma. *J Nucl Med* 1988;29:1038-1044.
27. Monsieurs M, Bacher K, Brans B, et al. Patient dosimetry for 131-I-Lipiodol therapy. *Eur J Nucl Med Mol Imaging* 2003;30:554-561.

1.1.2. ^{188}Re-HDD/Lipiodol Therapy for Hepatocellular Carcinoma: An Activity Escalation Study

^{188}Re-HDD/Lipiodol Therapy for Hepatocellular Carcinoma: An Activity Escalation Study

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ABSTRACT

The present study aimed to investigate the urinary excretion, organ dosimetry and toxicity following the intra-arterial administration of increasing activities of ^{188}Re-4-hexadecyl-1-2,9,9-tetramethyl-4,7-diaza-1,10-decanethiol/Lipiodol (^{188}Re-HDD/Lipiodol) for treatment of hepatocellular carcinoma (HCC) in patients with a well compensated cirrhosis. A secondary objective was to document the response. Methods: The activity levels were increased by 1.1 GBq/step after a 6 week interval without unacceptable adverse events in at least 5 subsequent patients. Three gamma-scintigraphies were performed up to 54 hours post-therapy. Absorbed doses to the various organs were calculated according to the MIRD-formalism, using the MIRDOSE 3.1 software. The toxicity was recorded until 6 weeks post-administration by means of the CTC-scale. The response was evaluated on magnetic resonance imaging (MRI) or computed tomography (CT) and by monitoring of alpha-fetoprotein (AFP). Results: Thirty-five treatments were carried out in 28 patients. Activities from 4.8 up to 7.0 GBq ^{188}Re-HDD/Lipiodol were administered via a transfemoral catheter. Urinary excretion eliminated (mean ± SD) 41.7 ± 9.7% of the injected activity within 46 ± 9.6 hours. The mean absorbed dose to the liver (including tumor) was 7.6 ± 2.2, 9.8 ± 4.9 and 15.2 ± 4.9 Gy for the 4.8, the 5.9 and the 7.0 GBq patient groups respectively, whereas the mean lung doses resulted in 5.3 ± 2.9, 6.8 ± 3.1 and 8.9 ± 4.5 Gy. The mean whole body doses for the increasing activity levels resulted in 0.6, 0.7 and 0.9 Gy. Treatment was well tolerated at all

activity levels, including the final level of 7.0 GBq. Further escalation of the administered activity was not feasible due to technical reasons, related to the radiolabeling procedure. Response assessment on MRI showed partial response, stable disease and disease progression in respectively 1, 28 and 2 assessable treatments. In 8 out of 17 treatment sessions with an initially elevated AFP a reduction ranging from 19-97% was observed 6 weeks later. Conclusion: Following the intra-arterial administration of 4.8 up to 7.0 GBq ^{188}Re-HDD/Lipiodol in patients with HCC and well compensated liver cirrhosis, no severe adverse events occurred. Further escalation was not feasible due to limitations in the radiolabeling procedure.

INTRODUCTION

Hepatocellular Carcinoma (HCC) is the most common type of primary liver cancer. HCC is among the most prevalent causes of cancer related deaths, in particular in South East Asia, Sub-Saharan Africa and Japan (*1*). In the West, its incidence keeps rising (*2,3*). The tumor often presents late and since more than 80% of patients presenting with HCC, suffer underlying cirrhosis, therapeutic possibilities are limited. Surgery, by either hepatectomy or liver transplantation, is the mainstay of curative treatment. Overall, if the degree of liver dysfunction and the tumor load is taken into consideration, the vast majority of patients is not eligible for surgery (*4*). Local strategies such as percutaneous alcohol injection and radiofrequent ablation are often applied in case of inoperable patients presenting with limited tumor load (*5,6*). For multifocal HCC, transarterial chemo-embolization is generally accepted and survival advantages were identified in patients with well compensated cirrhosis (*7*). Encouraging results using ^{131}I-Lipiodol have been reported, but radioprotective concerns regarding the use of ^{131}I have limited its widespread implementation (*8,9*). The long physical half-life of ^{131}I of 8 days and its high energetic gamma-ray of 364 keV leads to delayed hospitalization for radioprotection purposes. ^{131}I-Lipiodol therapy is generally well tolerated, but the use of activities exceeding 2.22 GBq is restricted by the above mentioned radioprotective issues.

Rhenium-188 (^{188}Re) has favorable characteristics for radionuclide therapy and considering the limited success of ^{131}I-Lipiodol for treatment of relatively large tumors, the switch towards a radionuclide with a higher energy of the beta-emission (2120 keV and 1960 keV for ^{188}Re versus 606 keV for ^{131}I) might yield improved response rates (*10*). ^{188}Re emits a gamma ray of 155 keV at an abundance of 15%, allowing gamma-camera imaging and it has a relatively short physical half-life of 17 hours, reducing radiation protection problems. Additionally, the radionuclide is eluted from a ^{188}W/^{188}Re-generator, which has a long useful shelf-life of several months and provides a good yield of carrier-free ^{188}Re routinely (*11*).

The first clinical results using ^{188}Re-4-hexadecyl-1-2,9,9-tetramethyl-4,7-diaza-1,10-decanethiol/Lipiodol (^{188}Re-HDD/Lipiodol) were reported by Sundram et al. (*12,13*). Whereas Sundram and coworkers applied activities varying from 1.8-9.8 GBq, depending on the dose estimations following the administration of a scout dose, Lambert et al. used a fixed activity of 3.6 GBq. Tolerance was excellent in patients with well compensated cirrhosis (Child-Pugh class A) and according to the dosimetric estimations, a further escalation of the activity seemed feasible (*14*). The aim of the present study was to administer increasing activities of ^{188}Re-HDD/Lipiodol and to assess the urinary elimination, organ dosimetry and toxicity. A secondary end point was response assessment.

MATERIALS AND METHODS

Synthesis and Quality Control of the Radio-conjugate

$^{188}W/^{188}Re$-generators were purchased from the IRE (Institut des Radio-Eléments, Fleurus, Belgium). Lyophilised kits containing a HDD-chelator complex (4-hexadecyl-1-2,9,9-tetramethyl-4,7-diaza-1,10-decanethiol) were provided by the Seoul National University Hospital and ^{188}Re-HDD/Lipiodol was synthesized as described earlier by Jeong et al. (*15,16*). The concentrated eluate (6 mL) from a commercially available $^{188}W/^{188}Re$-generator, was heated with the HDD/$SnCl_2$ kit at 100°C for 1 hour to produce ^{188}Re-HDD complex (*16,17*). 3 mL of Lipiodol was added and mixed on a vortex to extract the ^{188}Re-HDD into the Lipiodol. After centrifugation at 4000g the ^{188}Re-HDD/Lipiodol fraction was separated. Finally, the ^{188}Re-HDD/Lipiodol layer was washed with a 0.9% NaCl solution. Quality control was performed according to the method described by Jeong et al. by ITLC (ITLC-SG plates, Gelman Sciences, mobile phase: 0.9% NaCl and Acetone) (*15*).

Study Design

Groups of at least 5 patients were treated with increasing activities of ^{188}Re-HDD/Lipiodol, starting at 4.8 GBq. Escalation of the administered activity was implemented in increments of 1.1 (± 0.55) GBq, following an interval of 6 weeks without the occurrence of an unacceptable event in at least 5 consecutive patients. If a case of unacceptable toxicity was recorded in the first 5 patients, a minimum of 10 patients had to be treated at this activity level without the occurrence of an additional unacceptable adverse event. However, if a second case of unacceptable toxicity was observed, the study was stopped at that activity level.

An adverse event was considered unacceptable if it was grade 4 or 5 according to the CTC- scale (Common Terminology Criteria for Adverse Events, Version 3.0. Cancer Therapy Evaluation Program. June 10, 2003. http://ctep.cancer.gov) and at least "probably related to the investigational agent". However, grade 4 laboratory aberrations related to liver dysfunction could be considered acceptable depending on the baseline status of particular cases. On the other hand, grade 3 toxicity could be deemed unacceptable if it was prolonged or having a clear negative impact on the patient's quality of life, compared to the baseline status. Any increase of more than 2 points on the Child-Pugh classification for liver dysfunction lasting until week 6 was regarded unacceptable, unless tumor progression occurred.

Patients without evidence of disease progression on imaging were eligible for additional sessions with 12 week intervals. An individual patient always remained within the same activity level if the inclusion criteria were still met.

Patient Selection

The diagnosis of HCC had to be established by means of biopsy or conventional imaging techniques, such as Computed Tomography (CT)-scan, Magnetic Resonance Imaging (MRI) or arteriography, possably in conjunction with an elevated alpha-fetoprotein (AFP) level exceeding 400 ng/ml (*6*). For lesions smaller than 2 cm, a consensus had to be reached by two independent

radiologists. Exclusion criteria were: eligibility for liver resection, pregnancy and breast feeding, age <18 years, Child B or C status according to the modified Child-Pugh score, poor general condition (Karnofsky <70%), white blood cell count <2. 10^3/µl, forced expiratory volume in 1 second (FEV-1) or lung diffusion capacity for carbon monoxide (DLCO) <40% of the predicted value, abnormal baseline CT-scan of the chest, other active primary malignancy than HCC, symptomatic extra-hepatic metastasis, and potentially toxic anticancer treatment in the preceding 6 weeks (*18*). Additional contra-indications for the arteriography and isolation procedure were the following: serum creatinine level >2 mg/dl, prothrombin time <50 % of the normal value, platelet count <50. 10^3/µl, inability of self-care, encephalopathy and incontinence. The study was approved by the institutional ethics committee and an informed written consent was obtained from all patients.

Administration

Under local anesthesia, a 5 French catheter was inserted transfemorally and introduced into the arteria hepatica propria. After obtaining a diagnostic hepatic arteriogram, approximately 4 ml of ^{188}Re-HDD/Lipiodol was injected slowly into the into the arteria hepatica propria under fluoroscopic control. Whenever an aberrant arterial supply was present, the radio-conjugate was injected selectively into the right and/or left hepatic artery separately. The volume of 4 ml was divided over the different hepatic arteries proportional to the volume of liver parenchyma supplied by the respective artery. To reduce uptake of free perrhenate in the thyroid or gastric mucosa, patients received 1 gram sodium perchlorate for 3 days.

Assessment of Urinary Elimination and Organ Dosimetry

Following ^{188}Re-HDD/Lipiodol administration patients were hospitalized in a dedicated radionuclide therapy room for 3 days. During the hospitalization, patients collected their urine. Urine samples were analyzed in a NaI(Tl) 3”x3”gamma well counter calibrated for ^{188}Re (Cobra II, Perkin Elmer, USA). Time-activity curves were generated and fitted mono-exponentially (SPSS software version 10.0, Chicago, USA).

Whole body scintigraphies were acquired at ($\pm$ SD) 3.7 $\pm$ 1.1, 24.5 $\pm$ 3.0 and 49.9 $\pm$ 3.5 hours post-administration using a double or triple headed gamma camera (respectively AXIS® and IRIX®, Philips, Eindhoven, The Netherlands) equipped with medium energy parallel hole collimators. Scan speed varied from 30 to 10 cm/minute depending upon the time elapsed since administration. The imaging window was set at 155 keV (20%). For quantification purposes a syringe containing a known activity of ^{188}Re (mean activity $\pm$ SD: 16.0 $\pm$ 3.0 MBq) was included in the whole body scan. Regions of interests (ROI) were drawn around the syringe, the total body, the liver (including tumor), the lungs and a background region on the first scan. The background corrected geometric mean of the total counts in the ROIs was used to calculate the total amount of activity in these regions, using the known activity in the syringe and experimental factors determined on an anthropomorphic phantom (Alderson Heart/Thorax SPECT phantom) for the conversion of the syringe activity into organ activity. In the latter conversion factors, the attenuation and scatter

effects in this standard phantom were taken into account. The overall uncertainty using this methodology for the activity calculation was less than 18%.
Mono-exponential time activity curves were generated for the total body, the liver and lungs using SPSS 10.0 software. Source organ residence times were determined from integration of the time activity curves. Absorbed doses to the various organs were calculated according to the Medical Internal Radiation Dose (MIRD) formalism, using the MIRDDOSE© 3.1 (Oak Ridge Associated Universities, USA) software package (*19,20*). The patient's dose rate was regularly measured at one meter distance of the liver region using a survey meter.

Toxicity and Response Assessment
Laboratory testing of red and white blood cell counts, platelets, liver function and renal function were performed shortly before treatment, following 48 hours and 2 and 6 weeks post-injection. Clinical evaluation of toxicity was performed daily during hospitalisation and 2 and 6 weeks later. Lung function testing was performed before treatment and 6 weeks later. Toxicity measurements were scored by means of the CTC-scale. Laboratory findings were compared by means of Friedman and Wilcoxon statistical testing and the significance level was set at $P < 0.05$ (SPSS version 10.0). Radiological response was assessed by means of the RECIST (Response Evaluation Criteria in Solid Tumours) response criteria on CT-scans or MRI images acquired 6 weeks following treatment (*21*). AFP levels were measured at baseline, week 2 and week 6.

RESULTS

Between January 2004 and February 2005, 28 patients agreed to participate in the presented study. Nine patients underwent 14 treatment sessions with 4.8 GBq ^{188}Re-HDD/Lipiodol. In 1 patient his second administration was performed with only half of the planned activity due to a technical problem during the radiolabeling procedure. Eleven administrations with 5.9 GBq of the radioconjugate were carried out in 9 patients and 10 patients underwent a single treatment session with 7.0 GBq ^{188}Re-HDD/Lipiodol. Radiochemical purity was in all cases higher than 95%. The total radiochemical yield (n=34) was 49% ± 5.4 %.
All patients but 2 were Belgian. The mean age was 69 years (range 48-82 years). The sex-ratio was 22 males versus and 7 females. Among the 28 patients, only 5 patients had undergone previous anti-cancer treatment. Patient characteristics are summarized in Table 1. Okuda and Cancer of the Liver Italian Program (CLIP) scores are prognostic staging systems for HCC. Prognosis worsens with increasing scores (*22,23*). Five patients presented with a thrombosis of the portal vein, which is a negative prognostic factor.
The radio-conjugate was injected in the into the arteria hepatica propria, both right and left branch and only the right branch in respectively 15, 11 and 2 patients. In the latter 2 cases only the right lobe was affected and treatment of the left lobe was not feasible due to an aberrant vascular anatomy.

TABLE 1: Patient characteristics
Etiology: chronic hepatitis B infection (HBV), chronic hepatitis C infection (HCV).

	Number of patients
Etiology	
alcohol	10
HBV	4
HCV	10
idiopathic	2
hemochromatosis	1
auto-immune	1
Child-Pugh score	
no cirrhosis	1
5 points	23
6 points	4
Okuda score	
class I	27
class II	1
class III	0
CLIP score	
0 points	7
1 points	12
2 points	6
3 points	2
4 points	1
Karnofsky-index	
100%	17
90%	8
80%	3

Urinary Elimination and Organ Dosimetry

Urinary excretion was assessable during hospitalization in 25 treatments. A mean of 41.7 % (± 9.7 SD, range: 20.1-56.1) of the administered activity was excreted within 46 hours (± 9.6 SD, range: 20-57) following administration. On whole body scintigraphy uptake in the liver, lungs and bladder was seen. Occasional gastro-intestinal or thyroid activity was very faint. The organ absorbed doses as well as the whole body dose were calculated in 12 sessions with 4.6 GBq, 8 sessions with 5.8 GBq and 7 sessions with 6.8 GBq and estimates are summarized in Table 2.

The dose rate at one meter dropped below 20μSv/h within the first 48 hours following administration in all patients.

TABLE 2: Normal organ dosimetry
Mean absorbed dose estimations after treatment with 4.6 ± 0.3, 5.8 ± 0.3 and 6.8 ± 0.2 GBq ^{188}Re-HDD/Lipiodol respectively. LLI (lower large intestine), ULI (upper large intestine)

Target Organ	**Absorbed Dose (Gy)**								
	4.6 ± 0.3 GBq ^{188}Re-HDD/Lipiodol (n=12)			5.8 ± 0.3 GBq ^{188}Re-HDD/Lipiodol (n=8)			6.8 ± 0.2 GBq ^{188}Re-HDD/Lipiodol (n=7)		
	Mean	**SD**	**Range**	**Mean**	**SD**	**Range**	**Mean**	**SD**	**Range**
Liver	7.6	2.2	4.6 – 10.4	9.8	4.9	5.6 – 14.9	15.2	4.9	12.3 – 21.8
Lungs	5.3	2.9	2.0 – 10.3	6.8	3.1	3.8 – 8.9	8.9	4.5	5.6 – 14.7
ULI Wall	0.3	0.1	0.2 – 0.7	0.4	0.2	0.3 – 0.6	0.4	0.2	0.3 – 0.7
Kidneys	0.4	0.2	0.2 – 0.9	0.5	0.1	0.3 – 0.9	0.5	0.2	0.4 – 1.1
Stomach	0.3	0.1	0.2 – 0.4	0.4	0.1	0.3 – 0.5	0.5	0.2	0.3 – 0.6
LLI Wall	0.3	0.1	0.2 – 0.4	0.4	0.1	0.3 – 0.5	0.4	0.1	0.3 – 0.5
Thyroid	0.3	0.1	0.2 – 0.5	0.4	0.2	0.3 – 0.7	0.5	0.1	0.4 – 0.6
Red Marrow	0.3	0.1	0.2 – 0.4	0.4	0.1	0.3 – 0.5	0.4	0.1	0.3 –0.6
Whole body	0.6	0.1	0.4 – 0.7	0.7	0.2	0.5 – 0.9	0.9	0.3	0.6 – 1.2

Toxicity

Toxicity was assessable in 33 treatment sessions. One patient was lost of follow-up because he moved to another country and another patient was transplanted before assessment was complete. One additional patient was excluded from the statistical analysis since he was treated with only half of the planned activity ^{188}Re-HDD/Lipiodol due to a technical problem. In Table 3 the adverse events are scored according to the CTC-scale. Only the clinical events assumed to have at least a possible causal relation to the procedure and reflecting a worsening compared to the baseline values are tabulated.

Haematology. A small but statistically significant decrease in white blood cell counts was observed at week 6. Further analysis pointed out this was limited to the patients who underwent treatment with 5.9 and 7.0 GBq (*P*-values respectively 0.014 and 0.032). At these activity levels, respectively 4 and 1 patients developed grade 2 leukopenia at some time point following

treatment. Platelet counts did not change significantly. Values for white blood cells and platelets before therapy and on week 2 and 6 are depicted in Figure 1.

Liver Function. No clinical liver toxicity was encountered. In 8 sessions the Child-Pugh score was increased with a single point 6 weeks later. In half of these cases it concerned patients treated at the highest activity level. In one case an increase with 2 points was noticed but this patient suffered a rapidly rising AFP and an increase of the dimensions of his tumour. At discharge statistically significant increases in bilirubin and AST levels were recorded for all activity levels. Overall, an increase in bilirubin and AST of one CTC-grade was observed in respectively 12 and 6 therapies and these events did not occur more frequently at higher activity levels. Changes in liver parameters for the various activity levels are depicted in Figure 2.

Lung Function. No acute pulmonary symptoms were reported during the hospitalisation. Two patients developed pulmonary symptoms 2 to 5 weeks following the administration. One patient suffered a transient cough without fever 2 weeks following his second treatment session with 5.9 GBq. No deterioration of the lung function was seen 7 weeks later, nor abnormalities on high resolution CT-scan of the chest. The second case concerned a patient treated at the lowest activity level, who suffered an exacerbation of a pre-existing dyspnea and cough. This was definitely more severe after the second treatment compared to the first one and the second time it was accompanied by prolonged fever. Again, high resolution CT-scan of the lungs failed to show fibrotic changes. After oral steroid treatment, symptomatic relief was achieved but the recovery was complicated by an episode of gastro-intestinal bleeding. Lung function testing was regarded to be of limited value in this case because cooperation was not satisfying due to the abdominal discomfort. The relation to the investigational agent remains unclear since the patient had a history of episodes of unexplained fever and was known with chronic obstructive pulmonary disease. Statistical analysis by means of Wilcoxon testing (n=23 therapies) did not reveal any significant changes at week 6 compared to baseline pulmonary function, exception for the DLCO and pulmonary transfer factor for carbon monoxide (KCO) (*P*-values respectively 0.048 and 0.028, with a reduction of the overall mean of respectively 13 and 11%). However, 2 patients treated at the lowest activity level were unable to fully cooperate due to a recent gastro-intestinal bleeding and after exclusion of these cases, no statistically significant changes were observed. Changes in FEV-1, FVC (forced vital capacity), DLCO and KCO are illustrated in Figure 2.

Other. Three patients were admitted to hospital for gastro-intestinal bleeding (grade 3). Scintigraphy did not reveal extra-hepatic uptake of the radio-conjugate in these patients. CT-scans performed without contrast 2 weeks following treatment, did not show extra-hepatic Lipiodol. Taking into account their individual histories of variceal bleeding, the cause of these events was not considered to be likely related to the radionuclide therapy.

TABLE 3: Adverse events
Clinical adverse events are scored according to the CTC-scale version 3.0.

Time point	**Adverse event**	**Grade pre→post therapy**	**Number of therapies**	**Comment**
Week 1	fever	0 → 1	3	
	rash	0 → 1	3	related to contrast agent
Week 2	fatigue	0 → 1	2	
	pain (tumor)	0 → 2	2	rising AFP
	infection	0 → 1	3	without leukopenia
	cough	0 → 1	1	causal relationship unclear, no signs of infection, normal CT-scan of chest and lung function test
	cough, dyspnea, fever	0 → 2	2	twice in same patient, symptomatic relief with corticosteroids
Week 6	fatigue	0 → 1	4	
	arthritis and hyperuricemia	0 → 1	1	history of gout
	hemorrhage (esophagus/stomach)	0 → 3	2	ethyl abusus (1), corticosteroid treatment (1), history of variceal bleeding (2)
	hemorrhage (stomach)	0 → 1	1	administered activity too low, history of variceal bleeding

FIGURE 1: White blood cell counts (A-C) and platelets (D-F) before therapy and at week 2 and week 6. Bilirubin (G-I) and AST (J-L) levels before therapy, at discharge, week 2 and week 6. 4.8 GBq (left), 5.9 GBq (middle) and 7.0 GBq (right) ^{188}Re-HDD/Lipiodol.

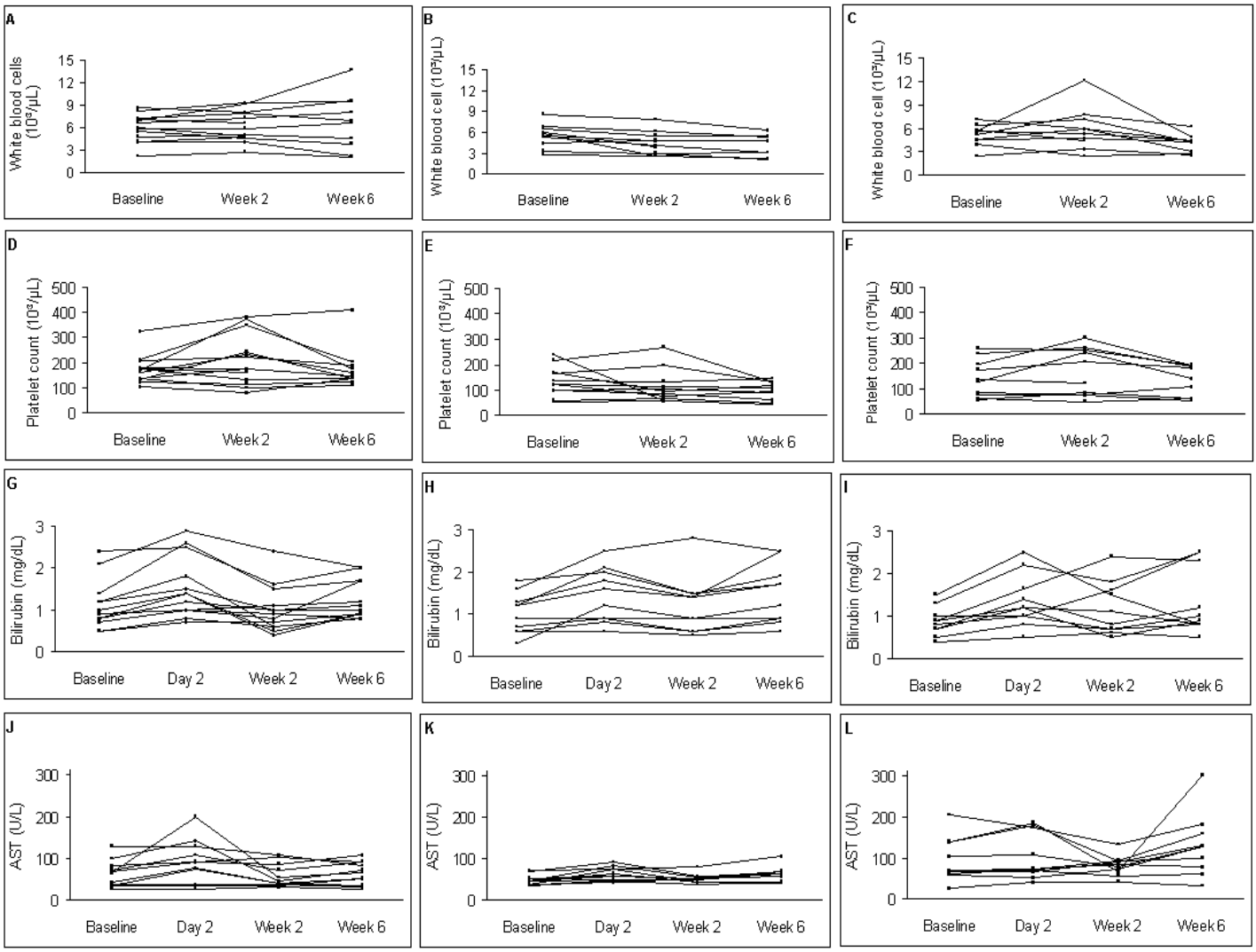
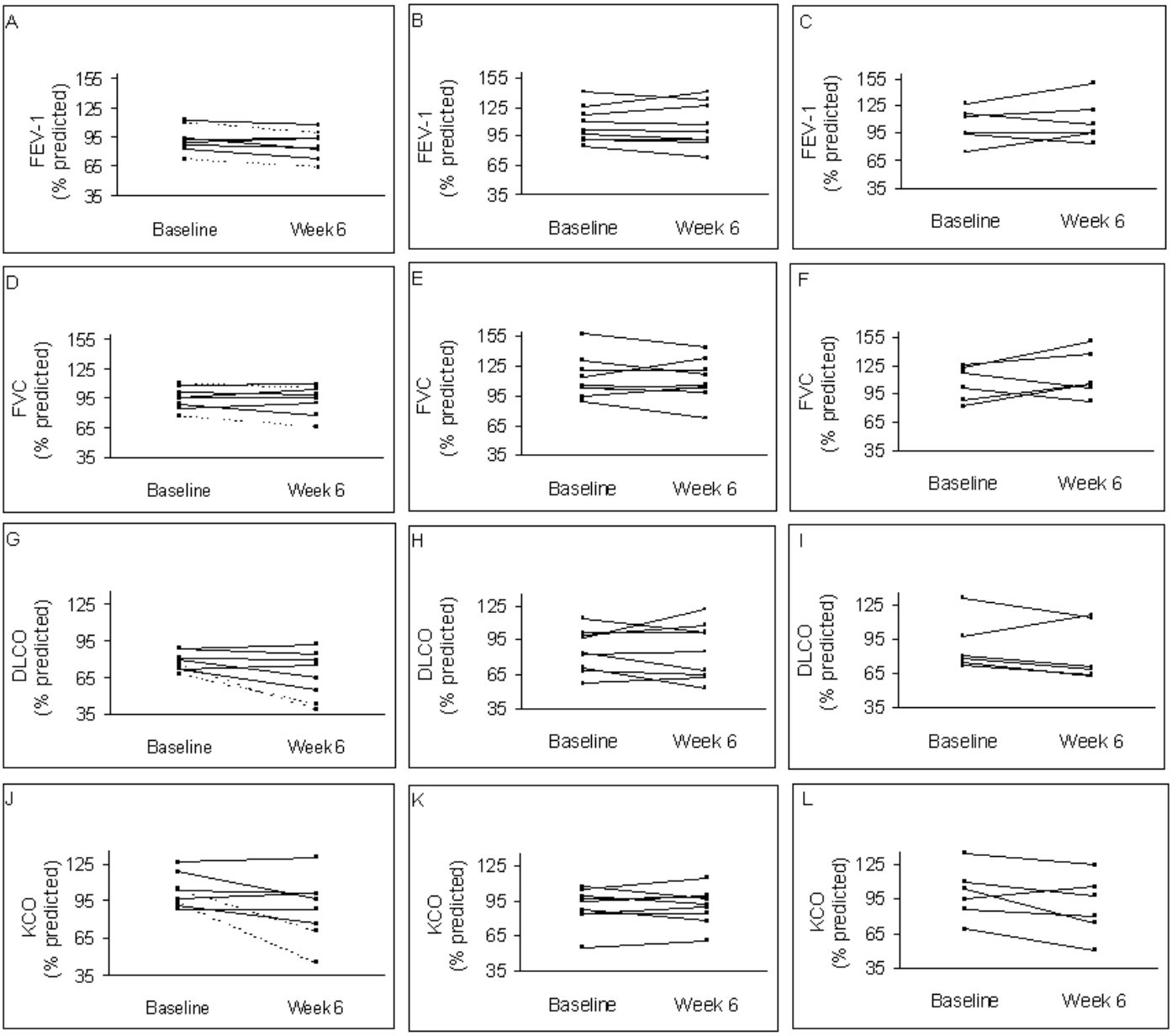


FIGURE 2: Changes in FEV-1 (A-C), FVC (D-F), DLCO (G-I) and KCO (J-L) before therapy and at week 6 for 4.8 GBq (left), 5.9 GBq (middle) and 7.0 GBq (right) ^{188}Re-HDD/Lipiodol. The measurements are expressed as percentage of the predicted value for that patient. The dotted lines represent patients who could not fully cooperate at the moment of lung function testing.
Forced expiratory volume in 1 second (FEV-1), lung diffusion capacity for carbon monoxide (DLCO), forced expiratory vital capacity (FVC), pulmonary transfer factor for carbon monoxide (KCO)

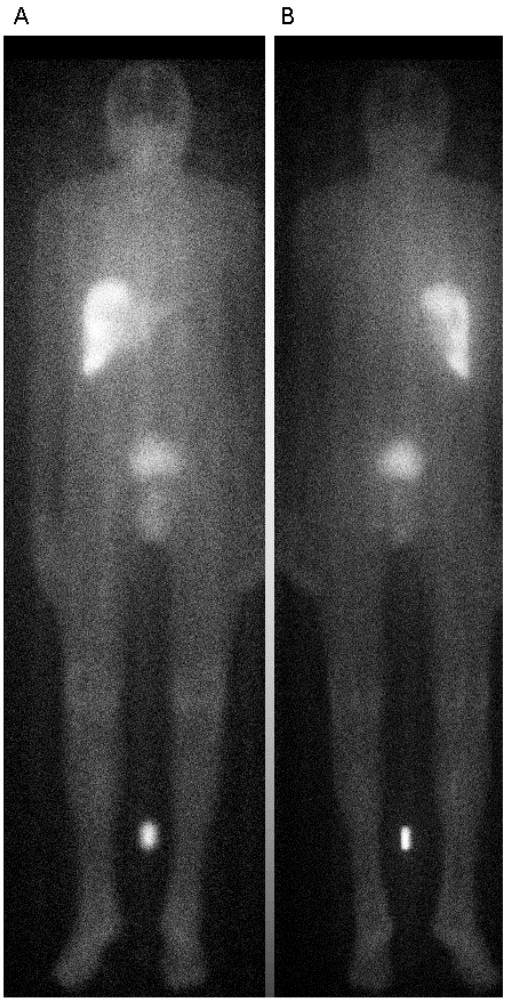


Response

Twenty five patients (31 treatment sessions) were assessable according to the RECIST-criteria. Two patients underwent a liver transplantation before response assessment was performed, 1 patient did not attend follow-up visits and in 1 case RECIST-criteria could not be applied. Overall, partial response, stable

disease and disease progression were observed in respectively 1, 28 and 2 treatments. The partial response was obtained in a 81 year old patient with underlying HBV-induced cirrhosis, treated with a single session of 4.8 GBq ^{188}Re-HDD/Lipiodol (Fig. 3). This patient had an unexpected low tumoral uptake of Lipiodol according to a CT-scan performed 2 weeks following his treatment. The response on imaging was accompanied by a steep decrease in AFP over 6 weeks (from 451 to 77 ng/ml).
One case of disease progression occurred in the patient whose second administration consisted of only half of the planned activity due to a problematic radiolabeling procedure.
In 8 out of 17 treatment sessions with an initially elevated AFP a reduction (median 47%, range 19-97% reduction) was observed 6 weeks later. Three patients had stable tumor markers whereas a rise in AFP (median 63%, 31-2012% increase) was documented in 6 therapies.

FIGURE 3: Whole body scintigraphy performed 24 hours post-administration of 4.8 GBq ^{188}Re-HDD/Lipiodol. Anterior (A) and posterior (B) image.



DISCUSSION

In the present study increasing activities of ^{188}Re-HDD/Lipiodol were administered. If it was technically feasible, we aimed at treating the entire liver since there is a high risk of developing metachronous HCC in the non-treated segments (*24*). Targeting the whole liver was technically feasible in all patients but 2. Concerning the labeling procedure, no major technical problems were encountered up to 7.0 GBq except in one treatment.

Instant thin-layer chromatography showed that the activity in the urinary samples was perrhenate (*14*). If compared to our earlier experiences, the use of increasing activities from 3.6 up to 7.0 GBq ^{188}Re-HDD/Lipiodol did not yield an altered relative urinary excretion (*P*-value: 0.30). If the shorter physical half-life of ^{188}Re is taken into account, the urinary excretion compares favorably with the observations using ^{131}I-Lipiodol (*25,26*). For all activity levels, organ dose estimates were well below the threshold levels for adverse radiation induced effects.

In the initial clinical experiences, Sundram et al. used 1.8-9.8 GBq ^{188}Re-HDD/Lipiodol, depending on the dose estimations following the administration of a scout dose and the activity was administered as close to the tumor feeding artery as possible. In this study, about 35 out of 70 patients did not experience any adverse event. The most frequent adverse events consisted of mild anorexia, right hypochondrial discomfort and low grade fever (*13*). Although we aimed at treating the whole liver, we had a similar observation. Irrespective of the relation with the radionuclide therapy, in 19 out of 32 assessable treatment sessions a symptom was reported by the patient. In 5 additional treatments, only laboratory changes were recorded. In 8 sessions, no changes compared to baseline were observed. Sundram et al. reported 2 cases of pleural effusion and these were attributed to radiation induced pneumonitis (*13*). In the present study, one patient suffered cough and dyspnea which exacerbated following her second treatment and required oral corticosteroid treatment. Similarities with a case previously described in the pilot study conducted earlier at our institution, are striking. The absorbed cumulative lung doses for both patients were estimated to be 10.4 and 11.5 Gy, hence too low to explain this evolution. Although is was not clear whether the occurrence of pulmonary symptoms was related to the ^{188}Re-HDD/Lipiodol therapy, we advocate that in the future particular attention is paid to patients developing pulmonary complaints.

The preliminary response rates point out that the vast majority of patients had stable disease according to the RECIST-criteria. However, these results are difficult to interpret since most patients were treated early after diagnosis and hence, no evidence of rapidly progressive disease could be established at inclusion. Moreover, extensive tumor necrosis may not be always be paralleled by a reduction in diameter of the lesion (*6*). In 2 out of 4 subsequently transplanted patients in this series over 99% of tumour necrosis was present in the explanted liver although assessment by imaging suggested disease stabilization.

Due to the relatively long half-life and high energy of the gamma-emission of ^{131}I, ^{131}I-Lipiodol treatment is associated with a hospital stay for up to 7 days in certain European countries. Besides the psychological burden for the patient, this isolation procedure is an extra cost. The need for rigorous radioprotective measures has been a major drawback for the administration of ^{131}I-Lipiodol activities exceeding 2.2 GBq. Moreover, for patients listed for liver transplantation, prior treatment with ^{131}I-Lipiodol excludes them from the waiting list for several weeks (*27*). In the present study, the patient's dose rate measured at 1 meter dropped below the local limit of 20 µSv/h within 48 hours following administration of ^{188}Re-HDD/Lipiodol, allowing a significant shorter hospitalization. Subsequent transplantation was allowed for after an interval of only 1 week. The shorter stay in the shielded therapy room and the use of on-site $^{188}W/^{188}Re$- generator improved the flexibility in treatment planning.

The labeling yield of ^{188}Re-HDD ranges between 50 and 70% and hence, the synthesis of activities exceeding 7.0 GBq routinely, poses a problem. A number of authors have focused on the development of new derivatives that may be produced at higher yields. These include bis-(diethyldithiocarbamato) nitrido ^{188}Re Lipiodol (^{188}ReN-DEDC) and ^{188}Re-$(S_2CPh)(S_3CPh)_2$ Lipiodol (^{188}Re-SSS Lipiodol). For intra-arterial ^{188}ReN-DEDC treatment, either alone or in combination with trans-arterial chemo-embolization, feasibility was shown in a series of 9 patients. However, some unexplained spleen and bone marrow uptake was observed 20 hours post-injection and 1 patient suffered grade 4 myelosuppression. Data on ^{188}Re-SSS Lipiodol in HCC patients are currently lacking (*28,29*).

Future work should focus on the optimization of the radiolabeling procedure of ^{188}Re-HDD/Lipiodol. Attempts should be made to elucidate various issues concerning the long term effects of cumulative treatment on the liver and lung tissue. In addition, efforts should be made in these studies to perform SPECT-tumor dosimetry.

CONCLUSION

Activities ranging from 4.8 up to 7.0 GBq ^{188}Re-HDD/Lipiodol were administered to 28 patients in 35 treatment sessions. Urinary excretion eliminated 41.7% (± 9.7) of the injected activity within 46 hours (± 9.6) after administration and did not differ significantly between the activity levels. The mean absorbed dose to the liver including the tumor was 7.6, 9.8 and 15.2 Gy for the 4.8, the 5.9 and the 7.0 GBq patient group respectively, whereas the mean lung doses resulted in 5.3, 6.8 and 8.9 Gy. The mean whole body doses for the different activity levels resulted in 0.6, 0.7 and 0.9 Gy. Treatment was well tolerated at all activity levels, without the occurrence of unacceptable adverse events. Further escalation of the administered activities was limited by technical reasons related to the radiolabeling procedure.

REFERENCES

1. Parkin DM, Bray F, Ferlay J, Pisani P. Estimating the world cancer burden: globocan 2000. *Int J Cancer* 2001;94:153-156.
2. El-Serag HB, Masson AC. Rising incidence of hepatocellular carcinoma in the United States. *N Eng J Med* 1999;340:745-750.
3. El-Serag HB, Davila JA, Petersen NJ, McGlynn KA. The continuing increase in the incidence of hepatocellular carcinoma in the United States: an update. *Ann Intern Med* 2003;139:817-823.
4. Schafer DF, Sorrell MF. Hepatocellular carcinoma. *Lancet* 1999;353:1253-1257.
5. Giovannini M, Elias D, Monges G, Raoul JL, Rougier P. Hepatocellular carcinoma. *Br J Cancer* 2001;84:74-77.
6. Bruix J, Sherman M, Llovet JM, et al. Clinical management of hepatocellular carcinoma. Conclusions of the Barcelona-2000 EASL conference. European Association for the Study of the Liver. *J Hepatol* 2001;35:421-430.
7. Llovet JM, Bruix J. Systematic review of randomized trials for unresectable hepatocellular carcinoma: chemoembolization improves survival. *Hepatology* 2003;37:429-442.
8. Raoul JL, Guyader D, Bretagne JF, et al. Prospective randomized trial of chemoembolization versus intra-arterial injection of 131-I-iodized oil in the treatment of hepatocellular carcinoma. *Hepatology* 1997;26:1156-1161.
9. Leung WT, Lau WY, Ho S, et al. Selective internal radiation therapy with intra-arterial iodine-131-lipiodol in inoperable hepatocellular carcinoma. *J Nucl Med* 1994;35:1313-1318.
10. Risse JH, Grünwald F, Kersjes W, et al. Intra arterial HCC therapy with ^{131}I-lipiodol. *Cancer Biother Radiopharm* 2000;15:65-70.
11. Knapp FF. Rhenium -188- A generator- derived radioisotope for cancer therapy. *Cancer Biother Radiopharm* 1998;13:337-349.
12. Sundram FX, Jeong JM, Zanzonico P, et al. Trans-arterial Rhenium-188 lipiodol in the treatment of inoperable hepatocellular carcinoma. An IAEA sponsored multi-centre phase 1 study. *World J Nucl Med* 2002;1:5-11.
13. Sundram F, Chau TC, Onkhuudai P, et al. Preliminary results of transarterial rhenium-188 HDD lipiodol in the treatment of inoperable primary hepatocellular carcinoma*. Eur J Nucl Med Mol Imaging* 2004;31:250-257.
14. Lambert B, Bacher K, Defreyne L, et al. ^{188}Re-HDD/Lipiodol Therapy for Hepatocellular Carcinoma: A Phase I Clinical Trial. J Nucl Med. *J Nucl Med* 2005;46:60-66.
15. Jeong JM, Kim YJ, Lee YS, et al. Lipiodol solution of a lipophilic agent, 188-Re-TDD, for the treatment of liver cancer. *Nucl Med Biol* 2001;28:197-204.
16. Lee YS, Jeong JM, Kim YJ, et al. Synthesis of ^{188}Re-labelled long chain alkyl diaminedithiol for therapy of liver cancer. *Nucl Med Commun* 2002;23:237-242.
17. Guhlke S, Beets AL, Oetjen K, et al. Simple new method for effective concentration of Re-188 solutions from alumina-based W-188-Re-188 generator. *J Nucl Med* 2000;4:1271-1278.

18. Pugh RN, Murray-Lyon IM, Dawson JL, Pietroni MC, Williams R. Transection of the oesophagus for bleeding oesophageal varices. *Br J Surg* 1973;60:646-649.
19. Loevinger R, Budinger TF, Watson EE. MIRD primer for absorbed dose calculations, New York, The Society of Nuclear Medicine, 1991.
20. Stabin MG. MIRDOSE: personal computer software for internal dose assessment in nuclear medicine. *J Nucl Med* 1996;37:538-546.
21. Therasse P, Arbuck SG, Eisenhauer EA, et al. New guidelines to evaluate the response to treatment in solid tumors. *J Natl Cancer Inst* 2000;92:205-216.
22. Okuda K, Ohtsuki T, Obata H, et al. Natural history of hepatocellular carcinoma and prognosis in relation to treatment. *Cancer* 1985;56:918-928.
23. (CLIP) investigators. A new prognostic system for hepatocellular carcinoma: a retrospective study of 435 patients: the Cancer of the Liver Italian Program. *Hepatology* 1998;28:751-755.
24. Brans B, Bacher K, Vandevyver V, et al. Intra-arterial radionuclide therapy for liver tumours: effect of selectivity of catheterization and 131I-lipiodol delivery on tumour uptake and response. *Nucl Med Commun* 2003;24:391-396.
25. Raoul JL, Bourguet P, Bretagne JF, et al. Hepatic artery injection of I-131-labeled lipiodol. Biodistribution study results in patients with hepatocellular carcinoma and liver metastases. *Radiology* 1988;168:541-545.
26. Nakajo M, Kobayashi H, Shimabukuro K, et al. Biodistribution and in vivo kinetics of iodine- 131 lipiodol infused via the hepatic artery of patients with hepatic cancer. *J Nucl Med* 1988;29:1066-1077.
27. Lambert B, Praet M, Vanlangenhove P, et al. Radiolabelled lipiodol therapy for hepatocellular carcinoma in patients awaiting liver transplantation: pathology of the explant livers and clinical outcome. *Cancer Biother Radiopharm* 2005;20:209-214.
28. Boschi A, Uccelli L, Duatti A, et al. A kit formulation for the preparation of 188Re-lipiodol: preclinical studies and preliminary therapeutic evaluation in patients with unresectable hepatocellular carcinoma. *Nucl Med Commun* 2004; 25:691-699.
29. Garin E, Noiret N, Malbert C, Lepareur N, et al. Development and biodistribution of ^{188}Re-SSS lipiodol following injection into the hepatic artery of healthy pigs. *Eur J of Nucl Med Mol Imaging* 2004;31:542-546.

1.1.3. ^{188}Re-HDD/Lipiodol for Treatment of Hepatocellular Carcinoma: A Feasibility Study in Patients with Advanced Cirrhosis

^{188}Re-HDD/Lipiodol for Treatment of Hepatocellular Carcinoma: A Feasibility Study in Patients with Advanced Cirrhosis

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ABSTRACT

This study aimed to investigate the feasibility of the intra-arterial administration of 3.7 GBq ^{188}Re-4-hexadecyl-1-2,9,9-tetramethyl-4,7-diaza-1,10-decanethiol/Lipiodol (^{188}Re-HDD/Lipiodol) for treatment of hepatocellular carcinoma (HCC) in patients with moderately advanced cirrhosis. Methods: Patients with HCC and underlying cirrhosis classified as Child-Pugh B in terms of severity were eligible. Whole body scintigraphies were performed at 4 time points post-injection. Absorbed doses to the various organs were calculated according to the MIRD-formalism. Urine was collected for 52 hours following injection. Toxicity was assessed until 6 weeks post-administration by means of the Common Toxicity Criteria for Adverse Events version 3.0 scale. Responses were evaluated on MRI and by alpha-fetoprotein (AFP) monitoring. Results: A mean activity (±SD) of 3.7 ± 0.2 GBq ^{188}Re-HDD/Lipiodol was administered in the hepatic artery to 12 patients. 36.2% ± 5.7 of the activity was excreted in the urine 52 hours post-injection. The mean absorbed dose to the liver, lungs, kidney and thyroid was respectively 7.6 ± 2.9 Gy, 4.8 ± 2.6 Gy, 0.8 ± 0.7 Gy and 0.2 ± 0.1 Gy. Two weeks following administration 6 out of 12 patients suffered from adverse events consisting of aggravations of pre-existing laboratory changes (3 patients), fatigue (2), vomiting (1), fever (1), encephalopathy (1) and ascites (1). Toxicity assessment at week 6 revealed single cases of worsening of hyperbilirubinaemia, pleural effusion, thrombocytopenia and dyspnea. Three patients dropped out because of deterioration of their general condition. The response was assessable by MRI in 8 patients: one case of partial response and 7 cases of stable disease were reported. Nine patients with an initially elevated AFP were evaluated. Stable

AFP was recorded in one patient and 3 showed a reduction, whereas a considerable increase was observed in 5 patients. Conclusion: Following the administration of 3.7 GBq ^{188}Re-HDD/Lipiodol, half of the Child-Pugh B patients in the present study suffered a worsening of their general condition or aggravation of pre-existing symptoms. This was associated with a rise in AFP in a considerable number of patients. In the future, administration of the radiopharmaceutical as close to the tumor feeding arteries as possible, might avoid further deterioration of the liver function and show enhanced antitumoral activity.

INTRODUCTION

Hepatocellular carcinoma (HCC) is a malignant epithelial tumor arising from parenchymatous liver cells (*1*). It is one of the world's most common malignancies, causing almost one million deaths annually (*2*). The major risk factor for HCC is cirrhosis. All types of cirrhosis predispose to HCC, but the incidence is particularly high in persistent infection with hepatitis B and hepatitis C, and in alcoholic liver disease (*3*). Only a minority of patients presenting with HCC fulfill the eligibility criteria for curative surgery, either partial liver resection or liver transplantation. Local ablative therapies such as Percutaneous Ethanol Injection (PEI), Radio Frequency Ablation (RFA), and Trans Arterial Chemo Embolization (TACE) have proven to be useful in selected patients and are mainly applied to offer palliation or as bridging strategies in patients awaiting liver transplantation (*4,5*).

Lipiodol is a mixture of iodized ethyl esters of the fatty acids of poppyseed oil. Injection of Lipiodol in the hepatic artery of HCC patients results in a selective and prolonged retention within the tumor. While Lipiodol alone does not appear to have any significant anti-cancer effect in hepatoma cells, radiolabeled Lipiodol has proven to be toxic for hepatoma cells (*6*). To date Lipiodol has been radiolabeled and clinically evaluated for the treatment of HCC respectively with Iodine-131 (^{131}I) and Rhenium-188 (^{188}Re). When compared to ^{131}I, ^{188}Re presents several advantages for radionuclide therapy. ^{131}I has a long physical half-life of 8.03 days and emits a high energetic gamma-ray of 364 keV, with an abundance of 82% necessitating delayed hospitalization for radioprotection purposes. ^{188}Re emits a gamma ray of 155 keV at an abundance of 15%, which allows for imaging and limits radiation protection problems. The maximum β-energy of ^{131}I is only 606 keV, whereas in ^{188}Re β-particles are emitted with a maximal energy of 2.1 MeV. As a result, the maximum range of the high-energy ^{188}Re β-emission is about 3 times longer compared to ^{131}I (maximum range in water: 2.9 and 10 mm for ^{131}I and ^{188}Re respectively) (*7*). Furthermore, the radionuclide is eluted from a ^{188}W/^{188}Re-generator, which has a long useful shelf-life of several months and provides a good yield of carrier-free ^{188}Re routinely (*8*).

The first clinical results using ^{188}Re-4-hexadecyl-1-2,9,9-tetramethyl-4,7-diaza-1,10-decanethiol/Lipiodol (^{188}Re-HDD/Lipiodol) were reported by Sundram et al. (*9,10*). The radiopharmaceutical was administered as close to the tumor feeding artery as possible and the administered activity was defined individually according to the findings following a scout dose administration. Tolerance was excellent. In a pilot trial conducted by Lambert et al. a fixed activity of 3.6 GBq ^{188}Re-HDD/Lipiodol was used and in patients with well compensated cirrhosis a further escalation of the activity seemed feasible (*11*). To date no studies have been conducted to define the tolerance of ^{188}Re-HDD/Lipiodol in patients suffering HCC with a moderately advanced underlying cirrhosis. The aim of the present study was to investigate the feasibility of ^{188}Re-HDD/Lipiodol treatment for patients suffering a Child-Pugh B degree of liver cirrhosis. A secondary end point was response assessment.

MATERIALS AND METHODS

Synthesis and Quality Control of the Radio-conjugate

$^{188}W/^{188}Re$-generators were purchased from the Oak Ridge National Laboratory, (Tennessee, USA) and the IRE (Institut des Radio-Eléments, Fleurus, Belgium). Lyophilised kits containing a HDD-chelator (4-hexadecyl-1-2,9,9-tetramethyl-4,7-diaza-1,10-decanethiol) were provided by the Seoul National University Hospital and ^{188}Re- HDD/Lipiodol was synthesized as described earlier (*12*). Briefly, the concentrated eluate (6 mL) from a commercially available $^{188}W/^{188}Re$-generator containing 11.1 GBq, was heated with the HDD/$SnCl_2$ kit at 100°C for 1 hour to produce ^{188}Re-HDD complex (*13*). 3 mL of Lipiodol was added and mixed on a vortex to extract the ^{188}Re-HDD into the Lipiodol. After centrifugation at 4000g the ^{188}Re-HDD/Lipiodol fraction was separated. Finally, the ^{188}Re-HDD/Lipiodol layer was washed with a 0.9% NaCl solution. Quality control was performed according to the method described by Jeong et al. by ITLC (ITLC-SG plates, Gelman Sciences, mobile phase: 0.9% NaCl and Acetone) (*14*). The total radiochemical yield was 50% ± 9 %. Radiochemical purity was in all cases higher than 95%.

Patient Selection

The diagnosis of HCC was established by means of biopsy or conventional imaging techniques such as Computed Tomography (CT)-scan, Magnetic Resonance Imaging (MRI) and arteriography, eventually in conjunction with a serum alpha-fetoprotein (AFP) level exceeding 400 ng/ml (*5*). For lesions smaller than 2 cm, 2 radiologists had to reach a consensus on the diagnosis of HCC. The severity of the underlying cirrhosis was assessed by clinical examination and laboratory testing one week before treatment. Only patients with a Child-Pugh B status according to the Child-Pugh scoring for cirrhosis were included in this study (Table 1) (*15*). The modified Child-Pugh classification system has been widely used in both alcoholic and nonalcoholic cirrhosis for assessment of severity of liver impairment and one-year mortality based on the degree of ascites and encephalopathy, serum concentration of bilirubin, albumin and prothrombin time. Child-Pugh grade A cirrhosis is considered as well-compensated disease whereas a Child-Pugh B score reflects a significant functional compromise. Patients suffering Child-Pugh C cirrhosis have decompensated disease with a median survival less than one year (*16*).

Exclusion criteria were: eligibility for liver resection, pregnancy and breast feeding, age <18 years, poor general condition (Karnofsky <70%), white blood cell count <1500/µl and potentially toxic anticancer treatment in the preceding 6 weeks. Contra-indications for the arteriography consisted of: serum creatinine level >2 mg/dl, prothrombin time <50 % of the normal value or platelet count <50 000/µl. In order to comply with the radioprotective guidelines, inability of self-care and incontinence were additional contra-indications. The study was approved by the institutional ethics committee and an informed written consent was obtained from all patients.

TABLE 1: Child-Pugh classification
5-6 points is scored as Child-Pugh A cirrhosis, 7-9 points is scored as Child-Pugh B and 10-15 as Child-Pugh C degree of cirrhosis.

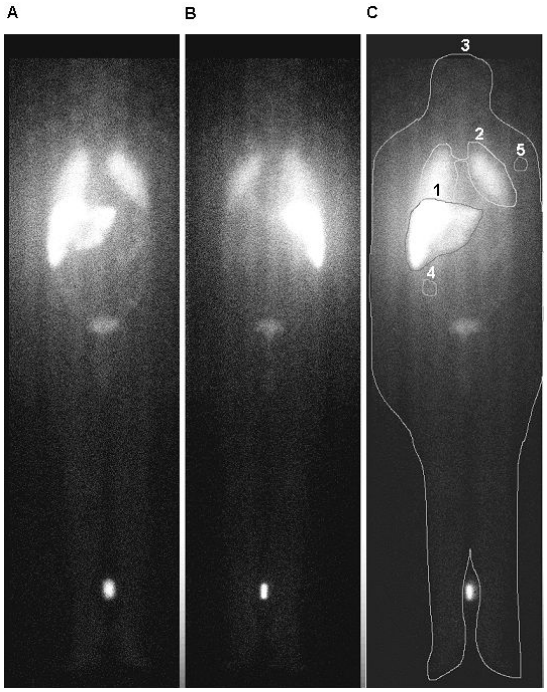
	1 point	**2 points**	**3 points**
Bilirubin (mg/dL)	<2	2-3	>3
Albumin (g/dL)	>3.5	2.8-3.5	<2.8
Ascites	no	slight	moderate
Encefalopathy	no	grade I- II	grade III-IV
Protrombin Time % INR	 >60 <1.7	 40-60 1.7-2.3	 <40 >2.3

Administration
Under local anesthesia, a 5 French diagnostic Cobra catheter was inserted transfemorally by Seldinger technique. First the portal vein was evaluated by catheterization of the superior mesenteric artery performing a mesentericoportography. Then a diagnostic hepatic arteriogram was obtained by catheterization of the coeliac trunc and common hepatic artery. Afterwards the diagnostic catheter or a microcatheter was introduced in the proper hepatic artery, and approximately 4 ml of ^{188}Re-HDD/Lipiodol was injected slowly under fluoroscopic control. Whenever aberrant arterial supply was present, the radio-conjugate was injected selectively into the right and left hepatic artery separately. The volume of 4 ml was divided over the different hepatic arteries proportional to the volume of liver parenchyma supplied by the respective artery. To reduce uptake of free perrhenate in the thyroid or gastric mucosa, patients received 0.5 gram sodium perchlorate orally prior ^{188}Re-HDD/Lipiodol administration followed by 1 gram daily until discharge.

Pharmacokinetic and Dosimetric Study
Following ^{188}Re-HDD/Lipiodol administration, patients were hospitalized in a dedicated radionuclide therapy room for 3 to 4 days. Four whole body scintigraphies (±SD) were acquired at 2.1 ± 1.7, 22.7 ± 1.2, 28.9 ± 2.1 and 60.1 ± 8.2 hours after administration, using a double or triple headed gamma camera (AXIS® respectively IRIX®, Philips, Eindhoven, The Netherlands) equipped with medium energy parallel hole collimators. The imaging window was set at 155 keV (± 20%). Scan speed varied from 30 to 10 cm/minute depending upon the time elapsed since administration. Regions of interests (ROIs) were drawn around the total body, the liver (including tumor), the lungs, a background region and a syringe containing a known activity of ^{188}Re (mean activity ± SD: 16.0 ± 1.9 MBq). The background corrected geometric mean of the total counts in the ROIs was used to calculate the total amount of activity in these regions, using the known activity in the syringe and experimental factors determined on an antropomorphic phantom (Alderson Heart/Thorax SPECT phantom) for the conversion of the syringe activity into organ activity (Figure 1). In the latter conversion factors, the attenuation and scatter effects in this standard phantom were taken into account. The overall

uncertainty using this methodology for the activity calculation was less than 18%. Mono-exponential time activity curves were generated for the total body, the liver and lungs using SPSS 10.0 software and source organ residence times were determined. Absorbed doses to the various organs were calculated according to the Medical Internal Radiation Dose (MIRD) formalism (*17*).
Urine was collected for 52 hours and samples were analyzed in a NaI(Tl) 3"x3"gamma well counter calibrated for ^{188}Re (Cobra II, Perkin Elmer, USA).
The patient's dose rate was regularly measured at 1 meter distance of the liver region using a survey meter.

FIGURE 1: Anterior (A) and posterior (B) view of a whole body scintigraphy (patient number 12) 28 hours following administration of 3.7 GBq ^{188}Re-HDD/Lipiodol. Part (C) illustrates the ROI-definitions on the geometric mean combination of the posterior and anterior view: liver (1), lungs (2), whole body (3), background to the liver (4), background to the lungs (5).



Toxicity and Response Assessment
Laboratory testing of red and white blood cells and platelets, liver function, renal function and serum AFP were performed one week before treatment, at discharge and 2 and 6 weeks post-injection. Laboratory findings were compared by means of Friedman statistical testing and the significance level was set at $P < 0.05$ (SPSS version 10.0). Clinical evaluation of toxicity was performed daily during hospitalization and 2 and 6 weeks later. Toxicity was scored by means of the Common Toxicity Criteria (Common Toxicity Criteria for Adverse Events, Version 3.0. Cancer Therapy Evaluation Program.). Radiological response was assessed by means of the RECIST response criteria on MRI images acquired 6 weeks following treatment (*18*). Patients without evidence of progressive disease were eligible for repetitive treatment sessions with a 12 week interval.

RESULTS

Twelve patients underwent a single treatment session. The mean administered activity per session was 3.7 ± 0.2 GBq (± SD). Patient characteristics are summarized in Table 2. The mean age was 63 years and the sex ratio was 9 males versus 3 females. Four patients presented with portal vein thrombosis (PVT) on arteriography. Okuda and Cancer of the Liver Italian Program (CLIP) scores are prognostic staging systems for HCC. Prognosis worsens with increasing scores (*19,20*).

Pharmacokinetic and Dosimetric Study

Urinary excretion was measured in all patients and 36.2% ± 5.7 (±SD) of the administered activity was excreted within 52 hours post-injection. Due to technical problems, data sets for calculation of the absorbed doses to the organs were complete in 6 out of 12 patients (Table 3). Effective half-lives for whole body, liver and lungs were respectively 15.1 ± 1.2 h, 16.2 ± 0.3 h and 16.4 ± 0.2 h. In all patients, the dose rate at 1 meter dropped below 20 µSv/h within the first 24 hours following administration and did not exceed 5µSv/h on day 2.

TABLE 2: Patient characteristics
Risk factors: chronic hepatitis C infection (HCV), alcohol (ALC), hemochromatosis (HC), none (NO). Recent previous treatment: none (NO), radionuclide therapy by means of ^{131}I-Lipiodol (I131) or ^{188}Re-HDD/Lipiodol (Re188), trans-arterial chemo-embolization (TACE). * Patients included in initial phase I study (*11*)

Nr	**Age** (years)	**Sex**	**Risk Factor**	**Child-Pugh score**	**Okuda score** (I-III)	**CLIP score** (0-6)	**PVT**	**Previous treatment** (interval in weeks)
1*	70	M	HCV	B9	II	2	NO	NO
2*	71	M	ALC	B9	II	3	NO	I131 (16 w)
3	60	M	NO	B7	I	2	NO	chemotherapy IV (22 w)
4	61	F	HCV	B9	II	3	NO	Resection (56 w)
5	48	M	ALC	B7	I	3	NO	tamoxifen
6	77	M	NO	B7	II	2	YES	NO
7	75	M	ALC	B8	II	3	YES	NO
8	59	F	HCV	B7	II	2	NO	NO
9	54	M	HC, ALC	B9	II	4	YES	NO
10	68	F	ALC	B7	I	4	YES	I131 (12 w)
11	44	M	HCV	B7	II	2	NO	TACE (22 w)
12*	72	M	HCV	B7	I	1	NO	Re188 (18 w)

TABLE 3: Mean absorbed dose estimations of 6 patients after treatment with 3.6 ± 0.2 GBq ^{188}Re-HDD/Lipiodol.
ULI (upper large intestine) LLI (lower large intestine)

Target Organ	Absorbed Dose (Gy)		
	Mean	SD	Range
Liver	7.6	2.9	4.6– 11.8
Lungs	4.8	2.6	1.7 – 10.4
ULI Wall	0.6	0.4	0.2 – 1.4
Kidneys	0.8	0.7	0.2 – 1.6
Stomach	0.3	0.1	0.1 – 0.4
LLI Wall	0.3	0.1	0.1 – 0.4
Thyroid	0.2	0.1	0.1 – 0.4
Red Marrow	0.3	0.1	0.1 – 0.4
Whole body	0.6	0.1	0.5 – 0.7

Toxicity

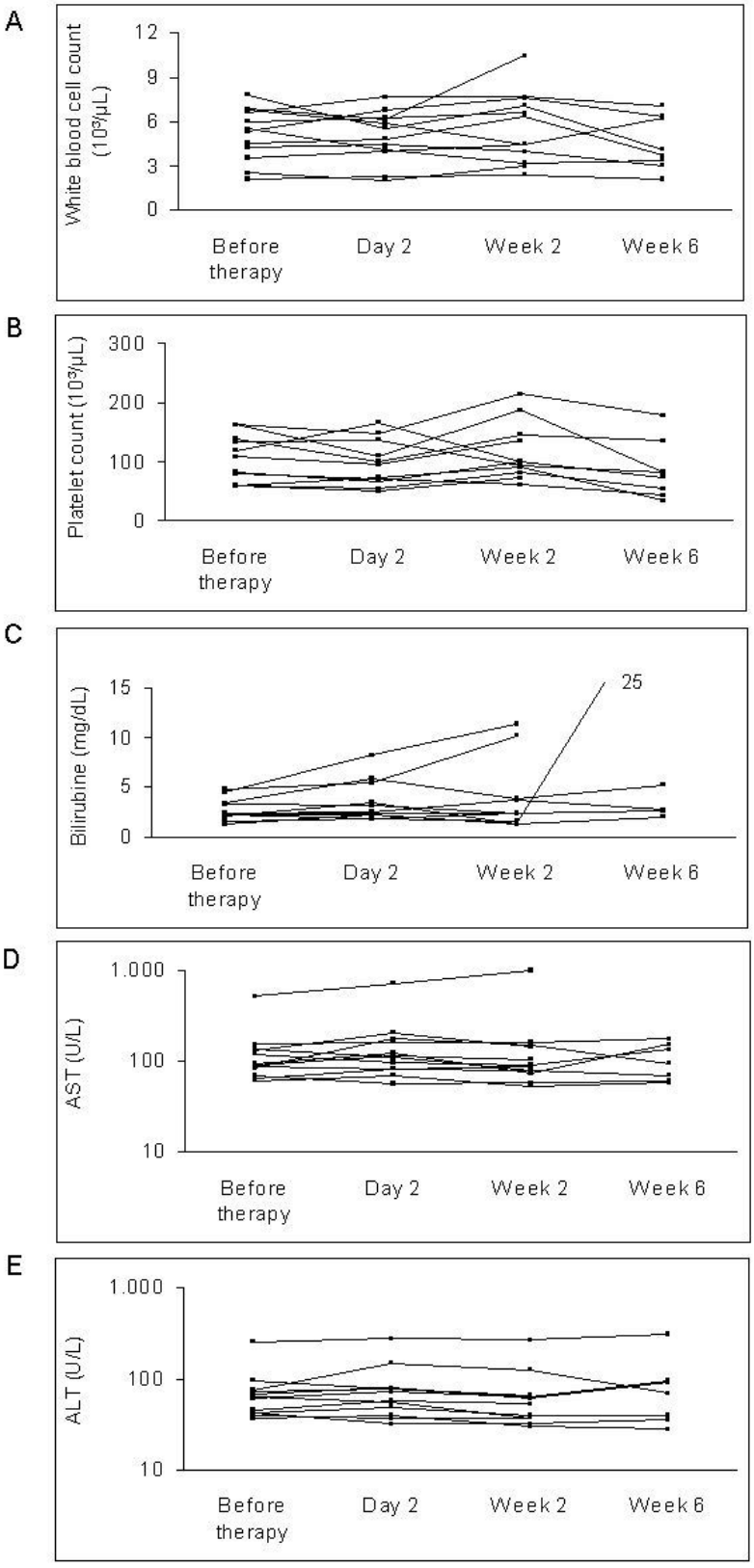
Table 4 summarizes the adverse events according to the Common Toxicity Criteria version 3.0. Only the events reflecting a worsening compared to the baseline values are tabulated. Four patients dropped out for response assessment 6 weeks following treatment: patient number 2 received a liver transplantation at week 5 and patients number 5, 7 and 10 did not attend follow-up visits at week 6 due to a worsening of their general condition. Seven patients experienced a severe adverse event, consisting of a grade 3 or 4 event. In three cases this event was associated with a rising tumor marker and thus the relation to the radiopharmaceutical remains unclear. Two patients suffered a severe aggravation of their jaundice. Patient number 10 developed rapidly progressive ascites at week 2. She could not attend further follow-up visits and died 8 weeks following treatment. Patient number 12 suffered mild dyspnea and coughing following his first treatment with ^{188}Re-HDD/Lipiodol, which was performed in the framework of a phase I pilot trial conducted earlier at our institution. After 18 weeks his tumor marker was rising and his cirrhosis had worsened to a Child-Pugh B degree, whereas he initially presented with a Child-Pugh A cirrhosis. A second treatment was performed in the framework of the present study and his pulmonary symptoms reoccurred. A complete relief of the symptoms was achieved with oral steroid treatment. However, the patient discontinued the medication and relapsed.

The most common adverse event during hospitalization consisted of a rise in aspartate amino transferase (AST) or alanine amino transferase (ALT). The early onset of this phenomenon suggests a relation with the radionuclide administration. Friedman statistical testing over the various time points however did not reach the level of significance (P <0.05) for any of the tested parameters (ALT, AST, gamma-glutamyl transferase, red blood cells, white blood cells, platelets, prothrombin time, albumin, total bilirubin and creatinine). Values for white blood cells, platelets, bilirubin, ALT and AST at baseline, discharge, week 2 and week 6 are depicted in Figure 2.

TABLE 4: Adverse events and changes in AFP level
Not applicable (NA)

Patient	Adverse event	Grade pre→ post therapy	Attribution to investiga-tional agent	Change in AFP
1	none			NA
2	fatigue (day 2) increase in bilirubin (week 2) increase in AST (day 2-week 2)	0 → 2 3 → 4 1 → 2	possibly possibly possibly	+30% (week 2); drop-out (week 6: transplanted)
3	fever (day 2-3) fatigue (week 2) increase in bilirubin (week 2) increase in bilirubin (week 6)	0 → 1 1 → 2 2 → 3 2 → 4	probably possibly possibly possibly	NA
4	vomiting (day 0) fever (day 0) AST elevation (day 2) hypoalbuminmia (day 2-week 2) ascites (week 6)	0 → 1 0 → 1 2 → 3 1 → 2 0 → 1	probably probably probably possibly possibly	+68% (week 6)
5	AST elevation (day 2-week 2)	3 → 4	possibly	NA drop-out (week 6: worsening general condition)
6	none			+69% (week 6)
7	encephalopathy (day 1)	0 → 2	possibly	+25% (week 2); drop-out (week 6: liver failure)
8	pleural effusion (week 6)	2 → 3	unlikely	-43% (week 6)
9	ALT elevation (day 2-week 6) AST elevation (day 2) AST elevation (week 2-week 6) increase in bilirubin (week 2)	1 → 2 1 → 3 1 → 2 1 → 2	possibly possibly possibly possibly	+37% (week 6)
10	ascites (week 2)	0 → 3	possibly	stable (week 2); drop-out (week 6: liver failure)
11	increase in bilirubin (week 6)	1 → 2	possibly	-63% (week 6)
12	leukopenia (week 2-week 6) thrombocytopenia (week 6) cough, dyspnea, pulmonary fibrosis (week 6) reduced carbon monoxide diffusion capacity	0 → 1 1 → 3 1 → 2 2 → 3	probably probably possibly possibly	-23% (week 6)

FIGURE 2: Changes in white blood cells (A), platelets (B), bilirubin (C), AST (D) and ALT (E) over time.



Response

Response on MRI. Four patients dropped out for response assessment. One patient (patient number 8) presented with 2 small tumors measuring 1.9 and 1.8 cm on MRI. On arteriography hypervascular blushes were obvious but CT-scan two weeks following Lipiodol administration failed to show prolonged uptake of Lipiodol in the lesions. In this patient a partial response was obtained with no evidence of HCC lesions on subsequent imaging. AFP dropped from 45 to 26 ng/ml. This patient underwent a liver transplantation 12 weeks following radionuclide therapy and no neoplastic lesions were found in the explant liver. The remaining 7 patients had stable disease.

Response on AFP. Changes in tumor marker are listed in Table 3. In 9 patients AFP levels were elevated prior to treatment. In 3 out of 9 treatment sessions a reduction in tumor marker (range 23-63%) was recorded 6 weeks later, but this was not observed in patients with baseline AFP-levels exceeding 400 ng/ml. Five patients showed a considerable rise in their tumor marker at follow-up visits 2 or 6 weeks following therapy.

DISCUSSION

Available literature concerning radionuclide therapy for HCC comprise reports of mixed patient populations of mainly Child-Pugh A and B patients and do not allow for conclusions focusing on Child-Pugh B patients in particular (*9,10,21,22*). Available data concerning other locoregional treatment strategies for HCC as well are limited to patients with a well compensated liver cirrhosis. In a meta-analysis conducted by Llovet et al. TACE with cisplatin or doxorubicin was reported to improve 2-year survival. (*23*) This publication confirmed TACE in its role of standard treatment for inoperable HCC. However, the randomized controlled trials taken into account in this meta-analysis consisted of 70 up to 100% Child-Pugh class A patients. Hence, its conclusions should be interpreted with caution if patients with advanced underlying liver dysfunction are considered. Best results were obtained in patients that underwent repeated TACE. Llovet and coworkers suggested that repeated treatment sessions are often only feasible in patients with a well preserved liver function, in whom the anti-tumoral effects are not offset by its toxic effects on the liver parenchyma. Although the present study design allowed for repeated ^{188}Re-HDD/Lipiodol administrations with 12 week intervals, the majority of the patients were not eligible for repeated treatment, due to a worsening of their general condition. These findings probably reflect a poor tolerance or lack of anti-tumoral effect in this patient group.

Sundram et al. reported 2 cases of pleural effusion and these were attributed to radiation induced pneumonitis (*10*). In our study patient number 12 developed grade 3 lung toxicity. The absorbed cumulative lung dose of was estimated to be 10.4 Gy, hence too low to explain this evolution. The timing of the occurrence of his symptoms and the associated bone marrow depression are suggestive for an increased sensitivity to ionizing radiation in this patient (*11*). Although a causal relationship is not clear, it is recommended that patients

developing pulmonary complaints following ^{188}Re-HDD/Lipiodol therapy are not eligible for repeated treatment sessions. Other adverse events in this series were related to underlying liver dysfunction and considering the features of this specific patient population, it is difficult to assess the cause of these adverse events: progressive underlying cirrhosis, tumor growth as well as toxicity due to the treatment may explain further liver decompensation. Even in patients with a rise in AFP, other factors than tumor progression might explain the occurrence of adverse events. A transient increase in AFP was documented following radiotherapy for HCC by Zeng and coworkers and was thought to reflect tumor repopulation. On the other hand, liver regeneration is a well known cause of an unspecific rise in AFP (*24*).

Urinary excretion in the present study was comparable with earlier findings in a group of patients with well compensated cirrhosis. ITLC analysis showed that the activity in the urinary samples was perrhenate (*11*). The absorbed doses to liver, lungs and thyroid were compared with the dose estimates obtained in a group of 14 Child-Pugh A patients treated with the same activity at our institution. No significant difference in dose estimates between Child-Pugh A and B patients were found (Mann-Whitney statistical test, SPSS version 10.0, $P < 0.05$) and hence, it is unlikely that the occurrence of adverse events was related to different distribution or elimination in Child-Pugh B patients.
In contrast to the initial experiences in patients with a well preserved liver function, the present findings concerning patients suffering moderately advanced cirrhosis do not support further evaluation of administration of 3.7 GBq ^{188}Re-HDD/Lipiodol in the proper hepatic artery or both left and right branch. A hyperselective approach, consisting of injecting the radiopharmaceutical as close to the tumor feeding artery as possible, might avoid further liver decompensation and induce an enhanced antitumoral effect. As the case for local ablative techniques like PEI and RFA, development of HCC lesions in untreated segments remains a risk in such a strategy (*23*). Therefore part of the activity could be given in the proper hepatic artery and the rest of the activity could be administered as hyperselective as possible. Future research should elucidate whether modified administration protocols yield better results.

CONCLUSION

Following the administration of 3.7 GBq ^{188}Re-HDD/Lipiodol in 12 patients suffering moderately advanced cirrhosis, 36.2% ± 5.7 of the injected activity was renally excreted within 52 hours post injection. The mean absorbed dose to the liver, lungs, kidney and thyroid was respectively 7.6 ± 2.9 Gy, 4.8 ± 2.6 Gy, 0.8 ± 0.7 Gy and 0.2 ± 0.1 Gy.
Treatment was well tolerated in 5 out of 12 patients. Seven patients suffered a worsening of their general condition, an aggravation of pre-existing symptoms or severe laboratory abnormalities. This was associated with a rise in AFP in a considerable number of patients. The results of the present feasibility study do not support further evaluation of the administration of 3.7 GBq ^{188}Re-HDD/Lipiodol in the proper hepatic artery or both right and left hepatic artery.

In the future, administration of the radiopharmaceutical as close to the tumor feeding arteries as possible, might avoid further deterioration of the liver function and show enhanced antitumoral activity.

REFERENCES

1. Röcken C, Carll-McGrath S. Pathology and pathogenesis of hepatocellular carcinoma. *Dig Dis* 2001;19:269-278.
2. Parkin DM, Bray F, Ferlay J, Pisani P. Estimating the world cancer burden: globocan 2000. *Int J Cancer* 2001;94:153-156.
3. Monto A, Wright TL. The Epidemiology and prevention of hepatocellular carcinoma. *Semin Oncol* 2001;28:441-449.
4. Schwartz JD, Schwartz M, Mandeli J, Sung M. Neoadjuvant and adjuvant therapy for respectable hepatocellular carcinoma: review of randomised trials. *Lancet Oncol* 2002;3:593-603.
5. Llovet JM, Burroughs A, Bruix J. Hepatocellular carcinoma. *Lancet* 2003;362:1907-1917.
6. Al-Mufti RA, Pedley RB, Marshall D, et al. In vitro assesment of Lipiodol-targeted radiotherapy for liver and colorectal cancer cell lines. *Br J Cancer* 1999;79:1665-1671.
7. ICRU. Stopping Powers for Electrons and Positrons. Report 37. Bethesda, MD: International Commission on Radiation Units and Measurements; 1984.
8. Knapp FF. Rhenium -188- A generator- derived radioisotope for cancer therapy. *Cancer Biother Radiopharm* 1998;13:337-349.
9. Sundram FX, Jeong JM, Zanzonico P, et al. Trans-arterial rhenium-188 Lipiodol in the treatment of inoperable hepatocellular carcinoma. An IAEA sponsored multi-centre phase 1 study. *World J Nucl Med* 2002;1:5-11.
10. Sundram F, Chau TC, Onkhuudai P, et al. Preliminary results of transarterial rhenium-188 HDD Lipiodol in the treatment of inoperable primary hepatocellular carcinoma*. Eur J Nucl Med Mol Imaging* 2004;31:250-257.
11. Lambert B, Bacher K, Defreyne L, et al. ^{188}Re-HDD/Lipiodol therapy for hepatocellular carcinoma: a phase I clinical trial. *J Nucl Med* 2005;46:60-66.
12. Lee YS, Jeong JM, Kim YJ, et al. Synthesis of ^{188}Re-labelled long chain alkyl diaminedithiol for therapy of liver cancer. *Nucl Med Commun* 2002;23:237-242.
13. Guhlke S, Beets AL, Oetjen K, et al. Simple new method for effective concentration of Re-188 solutions from alumina-based W-188-Re-188 generator. *J Nucl Med* 2000;4:1271-1278.
14. Jeong JM, Kim YJ, Lee YS, et al. Lipiodol solution of a lipophilic agent, 188-Re-TDD, for the treatment of liver cancer. *Nucl Med Biol* 2001;28:197-204.
15. Pugh RN, Murray-Lyon IM, Dawson JL, Pietroni MC, Williams R. Transection of the oesophagus for bleeding oesophageal varices. *Br J Surg* 1973;60:646-649.
16. Botta F, Giannini G, Romagnoli P, et al. MELD scoring system is useful for predicting prognosis in patients with liver cirrhosis and is correlated with residual liver function: a European study. *Gut* 2003;52:134-139.
17. Loevinger R, Budinger TF, Watson EE. MIRD primer for absorbed dose calculations, New York, The Society of Nuclear Medicine, 1991.

18. Therasse P, Arbuck SG, Eisenhauer EA, et al. New guidelines to evaluate the response to treatment in solid tumors. *J Natl Cancer Inst* 2000;92:205-216.
19. Okuda K, Ohtsuki T, Obata H, et al. Natural history of hepatocellular carcinoma and prognosis in relation to treatment. *Cancer* 1985;56:918-928.
20. (CLIP) investigators. A new prognostic system for hepatocellular carcinoma: a retrospective study of 435 patients: the Cancer of the Liver Italian Program. *Hepatology* 1998;28:751-755.
21. de Baere T, Taourel P, Tubiana JM, et al. Hepatic intraarterial 131I iodized oil for treatment of hepatocellular carcinoma in patients with impeded portal venous flow. *Radiology* 1999;212:665-668.
22. Raoul JL, Guyader D, Bretagne JF, et al. Prospective randomized trial of chemoembolization versus intra-arterial injection of 131-I-iodized oil in the treatment of hepatocellular carcinoma. *Hepatology* 1997;26:1156-1161.
23. Llovet JM, Bruix J. Systematic review of randomized trials for unresectable hepatocellular carcinoma: chemoembolization improves survival. *Hepatology* 2003;37:429-442.
24. Zeng ZC, Tang ZY, Yang BH, et al. Comparison between radioimmunotherapy and external beam radiation therapy for patients with hepatocellular carcinoma. *Eur J Nucl Med Mol Imaging* 2002;29:1657-1668.

1.2. Radiolabeled Lipiodol Therapy for Hepatocellular Carcinoma in Patients Awaiting Liver Transplantation: Pathology of the Explant Livers and Clinical Outcome

Radiolabeled Lipiodol Therapy for Hepatocellular Carcinoma in Patients Awaiting Liver Transplantation: Pathology of the Explant Livers and Clinical Outcome

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ABSTRACT

Background: Liver transplantation has become an important curative treatment option for hepatocellular carcinoma (HCC). Criteria for transplantation are strict and therefore it is crucial that patients awaiting transplantation do not suffer disease progression. One of the therapeutic options to achieve disease stabilization is neoadjuvant radiolabeled Lipiodol treatment. The present study aimed to document the drop-out rate on the waiting list, the pathological findings on the explant livers and the long term outcome of patients treated with radionuclide therapy while awaiting transplantation. Methods: Patients eligible for transplantation were treated with 2.1 GBq ^{131}I-Lipiodol or 4.1 GBq ^{188}Re-HDD/Lipiodol by transfemoral catheterisation of the hepatic arteries. Tumor necrosis was assessed in the explant livers and follow-up data such as drop-out from the waiting list, recurrence and survival following transplantation were retrospectively documented. Results: In 5 out of 22 explants necrosis exceeded 90%. Two patients died while on the waiting list (10%) and 4 out of 20 transplanted patients (20%) suffered recurrent disease. The overall recurrence free survival was 19.7 months (range 1.75-56) with a mean follow-up of 20.1 months. Conclusion: Our data support the evaluation on larger patient numbers to confirm the benefit of radiolabeled Lipiodol in candidates for liver transplantation suffering HCC.

INTRODUCTION

Hepatocellular carcinoma (HCC) is the most prevalent malignant primary liver tumor. It constitutes the third cause of cancer related deaths, responsible for more than 500 000 deaths worldwide annually (*1*). At present the mainstay of potentially curative strategy for HCC consists of hepatectomy, either a partial liver resection or a total removal of the liver, followed by cadaver organ transplantation or by living related liver donation. Three major criteria should be met, in case partial liver resection is considered: the absence of metastatic spread, no bilobar disease, and an adequate liver function afterwards (*2*). The two latter criteria are not essential in case transplantation is an option (*3*). As HCC typically presents late in its natural course, the vast majority of patients are not suitable for liver resection.

Theoretically, liver transplantation is the optimum therapy for HCC, as it provides removal of the primary tumor, as well as the cirrhotic parenchyma, which is a seed-bed for development of HCC. For advanced disease stages of HCC, retrospective analyses have shown poor outcomes after transplantation, yielding unacceptable recurrence rates (*3*). Therefore, strict criteria concerning the dimensions and numbers of tumors were advocated and in well selected patients survival rates equalled those obtained in patients transplanted because of benign liver pathology (*4-6*). Since tumor size is a very important variable in predicting recurrence, it is essential that patients are transplanted before progression occurs. The waiting time for a classic liver transplantation in the Western world may cause a delay which can allow the tumour to grow to stages that contraindicate transplantation and therefore several neoadjuvant strategies have been proposed (*7,8*).

Chemo-embolization is the most common neoadjuvant treatment but no randomized controlled studies have been reported so far to show an effect on the outcome (*8,9*). Using a non-selective arterial instillation, ^{131}I-Lipiodol has been found to be equally effective in tumor control but far better tolerated than the classical chemo-embolization by cisplatinum, Lipiodol and gelatin-sponge fragments in a group of inoperable, palliative patients (*10*). The initial studies using ^{131}I-Lipiodol in a neoadjuvant setting were conducted by Brans et al. and by Raoul et al. (*11,12*).

The present study aims to report our experiences using radiolabeled Lipiodol for disease stabilization in patients awaiting liver transplantation. The drop-out rate of patients on the waiting list despite neoadjuvant treatment was calculated. Pathological examination of the explant livers was performed in order to document anti-tumoral effects of therapy. Clinical follow-up data of the transplant patients were retrospectively collected and recurrence rates were estimated.

MATERIALS AND METHODS

Patient selection

The diagnosis of HCC was established on histological examination of biopsy specimens or on the radiological appearance of the lesions on imaging, eventually in combination with a rise in serum alpha-fetoprotein level. Patients were judged unresectable because of co-existing decompensated liver cirrhosis and/or multinodular HCC. Before listing for transplantation, metastatic spread was ruled out by bone scan, computed tomography scan of the chest and brain. Patients undergoing radiolabeled Lipiodol treatment gave written informed consent. Patients suffering Child C cirrhosis were not eligible for radionuclide treatment. Patients who had other anticancer treatment than radiolabeled Lipiodol in their history were excluded from the analysis.

Neoadjuvant treatment

The ^{131}I-labeled Lipiodol (Lipiocis^{TM}) was purchased at CIS bio international (Gif-sur-Yvette, France) and since 2002, ^{188}Re-HDD/Lipiodol was used. Lyophilized kits containing a HDD-chelator (4-hexadecyl-1-2, 9, 9-tetramethyl-4, 7-diaza-1,10-decanethiol) were provided by the Seoul National University Hospital and ^{188}Re-HDD/Lipiodol was synthesized as described earlier by Lee et al. (*13*). After selective hepatic catheterisation, a mean activity of 2.1 GBq ^{131}I or 4.1 GBq ^{188}Re in a total volume of 2-4 ml Lipiodol was instilled in the proper hepatic artery or in its right and/or left branches.

Pathology of the explanted livers

Total hepatectomy specimens were transected in 1 cm thick slices and examined by a senior pathologist. Macroscopically, sampling from the explanted liver was focused on nodules corresponding to a tumor localization on MRI and, routinely of larger sized regenerative nodules in the other segments. Diagnosis of HCC was made on histology, depending on lightmicroscopical characteristics of cellular growth pattern and nuclear atypia. The number of HCC lesions and their size, differentiation, percent of necrosis and presence of microvascular invasion was noted.

Assessment of long term outcome

Follow-up after transplantation was made by control of tumor markers and imaging every three months. Survival was calculated from the day of transplantation until the date of the last follow-up visit or until death.

RESULTS

Between June 1999 and June 2004 37 patients, with biopsy proven HCC or lesions suspect for HCC on radiology, were awaiting liver transplantation and received neoadjuvant radiolabeled Lipiodol treatment at the Ghent University Hospital (Figure1). Nine patients who received other anti-cancer treatment modalities prior to radionuclide therapy, were excluded from further analysis. Twenty-eight transplant candidates were exclusively pretreated by radiolabeled

Lipiodol. The mean age was 62 (range 24-72) years and the group consisted of 24 males versus 4 females. The vast majority suffered underlying viral hepatitis, and to a lesser extend alcohol induced cirrhosis. Three and 21 patients underwent one single administration of respectively ^{188}Re-HDD/Lipiodol and ^{131}I-Lipiodol. Two patients underwent 2 sessions of ^{131}I-Lipiodol and two patients received 3 ^{131}I-Lipiodol administrations. In 18 patients both lobes were treated independent from lesion localisation and in 10 patients only the affected lobe was treated. The median interval between the last radiolabeled Lipiodol administration and the transplantation was 101 days (range 32-320).

Drop-out ratio on the waiting list for transplantation

Two patients underwent a cadaveric transplantation without being listed according to the classic eligibility criteria (so called "special request" cadaveric transplantations) and 6 patients received a split liver from an adult living donor and therefore the number of patients at risk to drop out for transplantation was 20. The average waiting time for classic cadaveric transplantation was 5.8 months. Two out of 20 patients on the waiting list did not undergo liver transplantation because of death caused by non-oncologic reasons and thus the drop-out ratio was 10% overall or 1.7 % per month in the subgroup exclusively pretreated by radiolabeled Lipiodol.

Pathological examination of the explanted livers

The diagnosis of HCC was confirmed by histological examination of the explant in 22 out of 26 cases. In 4 explants the diagnosis of HCC could not be confirmed since no lesions suspect for neoplastic disease were found. In these 4 cases the diagnosis of HCC was based on the presence of hypervascular lesions on imaging which did not exceed the critical diameter of two centimetres and hence, the diagnosis is debatable and these patients were excluded from further analysis. Lightmicroscopical findings in 22 assessable explants are summarized in Table 1.

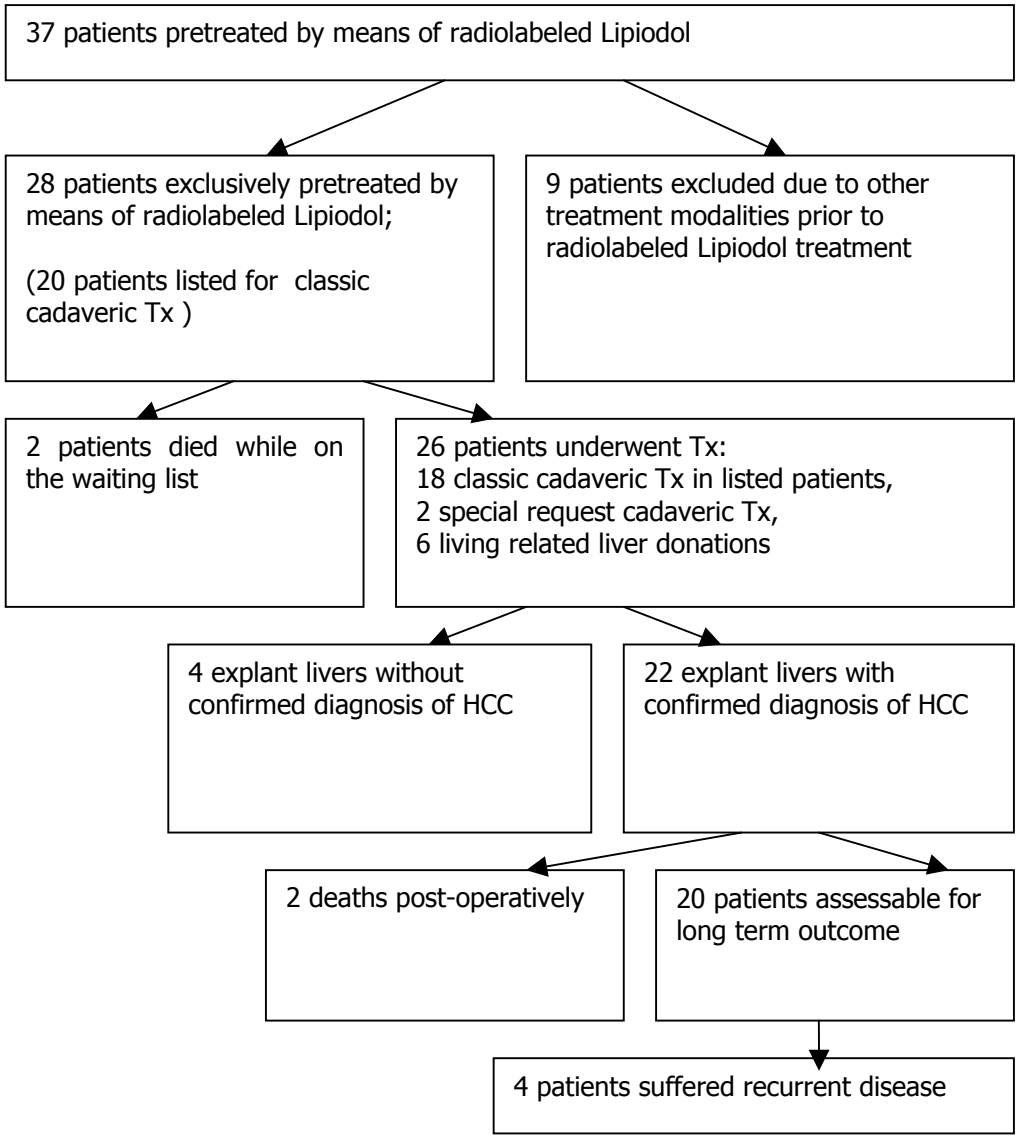
TABLE 1: Findings on pathology in explants with unequivocal diagnosis of HCC.

Mean number of tumors (range)	2.0 (1-7)	
Mean diameter of largest tumor (range)	3.0 cm (0.6-8.0)	
Tumor cell differentiation according to Edmondson's grading	I II III IV Not assessable	1 8 7 4 2
Necrosis (%) in largest lesion	0-50% >50-90% >90-100%	13 4 5
Presence of microvascular invasion	6/22	

Long term follow-up

Two patients died in the peri-operative phase due to surgical complications. Four out of 20 patients with histological diagnosis of HCC (20%), suffered recurrent disease after a mean disease free interval of 6.9 months (range 1.75-16). One patient suffered recurrent intrahepatic disease. Two patients developed pulmonary metastasis several months following transplantation and one patient was diagnosed with cutaneous metastasis. The overall recurrence free survival was 19.7 months (range 1.75-56) with a mean follow-up of 20.1 months. The estimated risk for recurrence was 11.9% per patient year. Six patients received a liver transplantation although their tumor load exceeded the criteria concerning size and number of the tumors described by Mazzaferro et al. and among these patients only one case of recurrent disease was observed (*4*).

FIGURE 1: Flowchart depicting the evolution of 37 patients treated by means of radiolabeled Lipiodol while awaiting liver transplantation (Tx).



DISCUSSION

Encouraging survival data on liver transplantation for treatment of early HCC have emerged the last decade. However, the shortage of donor organs and the increasing demands cause waiting times of more than 6 months in some parts of Europe and the USA. Several authors have shown that waiting times exceeding 6 months are associated with more than 15% rate of drop-out from the waiting list, and drop-out rates may reach 50% if expanded criteria are applied (*14*). In general, the risk for drop-out while awaiting liver transplantation is assumed to be about 4% per month (*15*). Even after correction for the 4 cases without histological diagnosis of HCC, a considerably lower drop-out ratio (2.2% per month or 12.5% over 5.8 months) was obtained in the present series.

Four out of 26 assessable explants (15%) did not reveal any neoplastic nor completely necrotic lesion. In these patients the pre-operative findings did not fulfil the classic diagnostic criteria of HCC, hence the diagnosis of HCC remains questionable. Earlier explant assessment reports have shown that false positive diagnosis despite state of the art imaging occurs in 9 up to 31% of transplant candidates depending on the tumor stage (*16*). It is unclear whether a complete response following radionuclide therapy occurred in our series or whether the exposition to ionizing irradiation should have been avoided. In 22 explants with clear evidence of HCC, at least 90% of the tumor was necrotic in 23% of the explants. This compares favorably with the findings in a historical control group, consisting of 19 patients transplanted at our institution with HCC without pre-treatment (n=12) or with an incidental finding of a HCC in the explant (n=7). Although these patients had comparable tumor characteristics, none showed 90% or more tumor necrosis.

The mean follow-time of 20.1 months is acceptable since most recurrences are expected to occur within the first year following surgery, and to a lesser extend the second year (*17*).
It is difficult to compare our series in terms of recurrence rates and survival with other reports since most series vary in patient characteristics and some series comprise incidental HCC, which is thought to have a better prognosis whilst others have focussed on more advanced disease stages (*6,12,14*). Reports dating from the early nineties often comprise patients with advanced disease stages and recurrence rates following transplantation were as high as 54%. More recent data suggest recurrence rates of 4-16% following transplantation, applying restrictive selection criteria (*17*). In our series less stringent criteria for transplantation were used and this is probably reflected in the recurrence rate of 20%. An additional important drawback for assessing the impact of strategies for patients awaiting liver transplantation, is the lack of controlled studies (*18*). Moreover, the long term results should be based on an intention-to-treat analysis and the true role of neoadjuvant treatment may be preventing drop-out in those patients who wait for long periods until transplantation (*8*).

No firm evidence for improved long term outcome to date is available for any adjuvant or neoadjuvant treatment strategy (*18*). Some evidence was established for the use of polyprenoic acid and ^{131}I-Lipiodol following partial liver resection in prospective randomized trials (*19,20*). In transplant patients, no firm evidence is provided yet and future research should attempt to elucidate the role of local, locoregional and systemic modalities. It is assumed that tumor cells are present in blood in microscopic quantities and therefore it seems reasonable to add systemic treatment during the immediate post-operative phase. Systemic treatment with limited toxicity, such as polyprenoic acid, are probably useful to test in a transplant patients at risk to relapse within the first year following surgery. Combined with aggressive local or locoregional treatment modalities whilst on the waiting list, this may yield an improved long term outcome if based on an intention-to-treat analysis.
Following ^{131}I-Lipiodol treatment patients were not eligible for surgery for 4 weeks because of radioprotection issues related to the relatively long physical half-life of ^{131}I (8 days). In patients with aggressive tumors, repeated radionuclide therapies were indicated and this lead to unacceptable long intervals during which the patients were not eligible for curative treatment. Because of the high energy of the gamma ray emission of ^{131}I (365 keV) and its physical half-life of 8 days, a hospitalization up to 7 days is indicated according to the local radioprotective legislation. Considering the limited success of ^{131}I-Lipiodol for treatment of relatively large tumors, the switch towards ^{188}Re, a radionuclide with a higher energy of the beta-emission (2120 keV and 1960 keV for ^{188}Re versus 606 keV for ^{131}I) might yield improved response rates. Since November 2002, we switched to ^{188}Re-HDD/Lipiodol and the use of ^{188}Re, with its physical half-life of 17 hours, avoids most of the radioprotection problems and allows a shorter hospitalization as well as further dose escalations (*21*). Following ^{188}Re-HDD/Lipiodol treatment, patients were eligible for transplantation after an interval of only one week.

CONCLUSIONS

The drop-out ratio in this patient group exclusively pretreated by means of radiolabeled Lipiodol, was 10% and after correction for cases without unequivocal diagnosis of HCC on the explant liver, a drop-out ratio from the waiting list of 12.5% or 2.2% per month was calculated.
On pathology nearly complete or complete necrosis was observed in 23 % of assessable explants. Four out of 20 patients (20%) suffered recurrent disease. The overall recurrence free survival was 19.7 months (range 1.75-56) with a mean follow-up of 20.1 months. Our data support the evaluation on larger patient numbers to confirm the benefit of radiolabeled Lipiodol in candidates for liver transplantation suffering HCC.

REFERENCES

1. Parkin DM, Bray F, Ferlay J, Pisani P. Estimating the world cancer burden: Globocan 2000. *Int J Cancer* 2001;94:153-156.
2. Schafer DF, Sorrell MF. Hepatocellular carcinoma. *Lancet* 1999;353:1253-1257.
3. Little SA, Fong Y. Hepatocellular Carinoma: Current surgical management. *Semin Oncol* 2001;28:474-486.
4. Mazzaferro V, Regalia E, Doci R et al. Liver transplantation for the treatment of small hepatocellular carcinoma in patients with cirrhosis. *N Eng J Med* 1996;334:697-671.
5. Figueras J, Jaurrieta E, Valls C et al. *Hepatology* 1997;25:1485-1489.
6. Haug CE, Jenkins RL, Rohrer RJ et al. Liver transplantation for primary hepatic cancer. *Transplantation* 1992;53:376-382.
7. Llovet JM., Burroughs A, Bruix J. Hepatocellular Carcinoma. *Lancet* 2003;362:1907-1917.
8. Wall WJ, Marotta PJ. Surgery and Transplantation for Hepatocellular Cancer. *Liver Transplant* 2000;6:S16-22.
9. Llovet JM, Mas X, Aponte JJ et al. Cost effectiveness of adjuvant therapy for hepatocellular carcinoma during the waiting list for liver transplantation. *Gut* 2002;50:123-128.
10. Raoul JL, Guyader D, Bretagne JF et al. Prospective randomized trial of chemoembolization versus intra-arterial injection of 131-I-Iodized oil in the treatment of hepatocellular carcinoma. *Hepatology* 1997;26:1156-1161.
11. Brans B, De Winter F, Defreyne L et al. The anti-tumoral activity of neoadjuvant intra-arterial 131I-Lipiodol treatment for hepatocellular carcinoma: a pilot study. *Cancer Biother Radiopharm* 2001;16:333-338.
12. Raoul JL, Messner M, Boucher E, Bretagne JF, Campion JP, Boudjema K. Preoperative treatment of hepatocellular carcinoma with intra-arterial injection of I131-labelled Lipiodol. *Br J Surg* 2003;90:1379-1383.
13. Lee YS, Jeong JM, Kim YJ et al. Synthesis of 188Re-labelled long chain alkyl diaminedithiol for therapy of liver cancer. *Nucl Med Commun* 2002;23:237-342.
14. Fisher RA, Maluf D, Cotterell AH et al. Non-resective ablation therapy for hepatocellular carcinoma: effectiveness measured by intention-to-treat and drop-out from liver transplant waiting list. *Clin Transplant* 2004;18:502-512.
15. Sarasin FP, Majno PE, Llovet JM, Bruix J, Mentha G , Hadengue A. Living donor liver transplantation for early hepatocellular carcinoma: A life-expectancy and cost-effectiveness perspective. *Hepatology* 2001;33:1073-1079.
16. Wiesner RH, Freeman RB, Mulligan DC. Liver transplantation for hepatocellular carcinoma: The impact of the MELD allocation policy. *Gastroenterology* 2004;127:S261-267.
17. Nonami T, Isshiki K, Katoh H et al. The potential role of postoperative hepatic artery chemotherapy in patients with high risk hepatomas. *Ann Surg* 1991;213:222-226.

18. Schwartz M. Liver transplantation for hepatocellular carcinoma. *Gastroenterology* 2004;127:S268-276.
19. Muto Y, Moriwaki H, Ninomiya M, et al. Prevention of second primary tumors by an acyclic retinoid, polyprenoic acid, in patients with hepatocellular carcinoma. *N Eng J Med* 1996;334:1561-1567.
20. Lau WY, Leung TW, Ho SK, et al. Adjuvant intra-arterial iodine-131-labelled Lipiodol for resectable hepatocellular carcinoma: a prospective randomised trial. *Lancet* 1999;353:797-801.
21. Lambert B, Bacher K, Defreyne L et al. 188Re-HDD/Lipiodol therapy for hepatocellular carcinoma: a phase I clinical trial. *J Nucl Med* 2005;46:60-66.

Preclinical research

2.1. Screening for supra-additive effects of cytotoxic drugs and gamma irradiation in an in vitro model for hepatocellular carcinoma

Screening for supra-additive effects of cytotoxic drugs and gamma irradiation in an in vitro model for hepatocellular carcinoma

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ABSTRACT

Hepatocellular carcinoma (HCC) is one of the most common malignancies in the world. A wide variety of treatment modalities are available for palliative therapy of HCC, although there is no strong evidence that these treatments can have a significant impact on survival. The aim of this work was to screen cytotoxic drugs, relevant in the treatment of HCC, for enhancement of the effect of irradiation in an *in vitro* model. As the majority of patients presenting with HCC suffer a reduced liver function, attention was paid to low dose effects of the cytotoxic drugs tested.
Multicellular tumor aggregates or "spheroids" of HepG2-cells were cultured and exposed to gamma irradiation in combination with cisplatin, gemcitabine or 5-fluorouracil. Toxicity was evaluated by means of comparative growth curves, an outgrowth assay and histology. Supra-additive effects lasting for 4 weeks were observed for all drugs tested in combination with a gamma irradiation of 10 Gy.

INTRODUCTION

Hepatocellular Carcinoma (HCC) is the most frequently occurring malignant tumor in the liver. It is the fifth most common malignancy in men, and the eighth most common in women worldwide (*1*). The high incidence of this malignancy in sub Sahara Africa and Southeast Asia can be explained by the hepatitis B virus being endemic in these regions. In North America and Europe, the main risk factors are chronic Hepatitis C infection and alcohol intake. Over 80% of patients presenting with HCC suffer from cirrhosis and the reduced liver reserve restricts treatment options in this patient group (*2*). A cure can be achieved through partial liver resection or transplantation, although the former is only feasible in less than 30% of patients and the latter is restricted by a shortage of donor organs (*3*). A wide variety of locoregional or systemic treatment modalities are available in a palliative setting, but there has been little evidence of a significant impact on survival (*4*). As HCC is a chemo- and radioresistant tumor, occurring often in patients that already have a reduced liver function, a combination of treatment modalities might improve outcome and minimize toxicity (*5*) .

Locoregional radionuclide therapy by means of intra-arterial injection of Lipiocis™ (Schering, Gif sur Yvette, France), a fatty acid mixture labeled with Iodine-131 (E β max: 606 keV, E γ: 364 keV, T1/2 phys: 8 days), has yielded promising results (*6*). Phase I clinical studies are being conducted to investigate the benefit of adding systemic to locoregional irradiation by Lipiocis™. Cisplatin has radiosensitizing capacities and has been shown to have cytotoxic effects in monotherapy for HCC (*7*).

The objective of this study was to determine whether cytotoxic drugs used for the treatment of HCC could enhance the effect of irradiation *in vitro*. The majority of patients that present with HCC suffer a reduced liver function, so particular attention was paid to low dose effects of the cytotoxic drugs tested. To reflect the *in vivo* situation, multicellular tumor aggregates or "spheroids" were cultured. Spheroids consist of different subregions, varying in the availability of oxygen, nutrients, pH, lactate and other substances. These factors influence the metabolic and proliferative status of the cells, resulting in a viable rim with proliferating cells and less mitotic activity in the deeper layers. Sutherland et al. reported that in general most of the proliferating cells are located in the outer three to five cell layers (75 µm) whilst quiescent cells are located more centrally at a depth of 100-220 µm (*8*). These often prove viable when removed from the deprived environment. Cells at a depth exceeding 160 µm may become necrotic. As spheroids consist of cell populations with variations in their degree of radio-resistance and cell cycle status, this model reflects the *in vivo* situation, such as micrometastasis or microregions within a vascularized tumor, more accurately than monolayers (*9*).

MATERIALS AND METHODS

A human cell line, HepG2, derived from a HCC was used to culture the spheroids (Bioreliance Ltd., Stirling, Scotland.) After trypsinisation of a

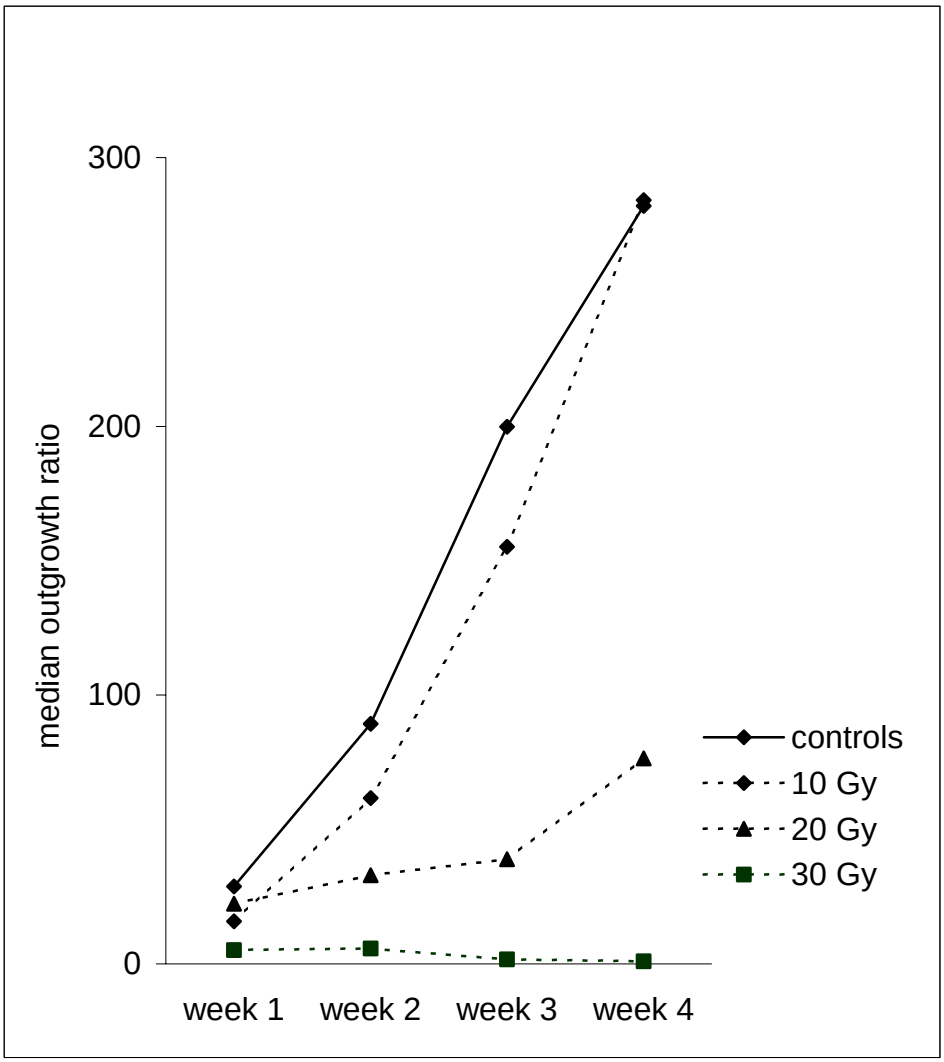
monolayer, spheroids were cultured by continuous gyratory shaking at 70 rpm (Heidolph Instruments, Schwabach, Germany) of a single HepG2-cell suspension in DMEM medium supplemented with 10% fetal bovine serum (Gibco, Invitrogen Corporation, Merelbeke, Belgium) and 5% CO2 at 37°C in PETG-flasks (Gosselin, Haezebrouck, France). After 7 to 10 days of shaking the spheroids attained a mean diameter of 189 ± 51 µm.
The spheroids were irradiated at 37°C with Cobalt 60 at a dose rate of 1 Gy per minute. Between 10 and 30 Gy were delivered. Three cytotoxic drugs were used at several concentrations: cisplatin at 0.1, 0.5 and 1 µg/ml (Platosin, Pharmachemie, Haarlem, The Netherlands), gemcitabine at 3 and 30 ng/ml (Gemzar, Eli Lilly, Fegersheim, France) and 5-fluorouracil at 50 and 100 µg/ml (Fluracedyl, Pharmachemie, Haarlem, The Netherlands). For combined modality experimental conditions, a fixed dose of 10 Gy was chosen.
The induced toxicity was evaluated by measuring the outgrowth potential of individual spheroids over 4 weeks. Within 24 hours of treatment, individually selected spheroids were placed in 24 well plates coated for cell culture (Becton Dickinson Biosciences, Erembodegem, Belgium). The longest diameter and its perpendicular are measured using a calibrated ocular micrometer and light microscopy. After some days a monolayer, varying in surface area and density, is formed by motile and clonogenic cells that surround the original spheroid. The longest diameter of the confluent outgrowth surface and its perpendicular are measured at 7 day intervals for one month and the outgrowth ratio is calculated by dividing the average diameter by the average diameter measured on the day of plating. The outgrowth ratios of treated spheroids are compared with controls and those exposed to combined treatment to those treated by monotherapy using Mann-Whitney statistics. In the case of severe damage to the spheroid caused by the experimental conditions, no adherence or a reduced monolayer surface or so-called outgrowth surface was observed. An outgrowth ratio of one signifies a lack of adherence and a reduced outgrowth potential reflects diminished cell motility and clonogenicity (*10*).
Growth was also assessed in a three-dimensional model, in which spheroids are individually transferred to an elisa-plate (Kima, Piove Di Sacco, Italy). As these plates do not permit adherence of the HepG2-spheroid, the growth pattern remains spherical in three dimensions. The longest diameter and its perpendicular are measured the day of plating and for one week thereafter. Some spheroids were selected after three days for histology and morphology was assessed by means of hematoxyllin and eosin staining.

RESULTS

Sensitivity of HepG2-spheroids to gamma irradiation

Absorbed doses of 10, 20 and 30 Gy were delivered to the spheroids with Cobalt 60-gamma irradiation. A dose of 10 Gy initially induced a reduction in the outgrowth of 55% relative to control spheroids but outgrowth fully recovered within 4 weeks. Absorbed doses of 20 Gy and 30 Gy caused the outgrowth to be only 27% and 1% respectively of that seen in the controls at week 4. In Figure1 the median outgrowth ratios are represented over time.

FIGURE 1: Median outgrowth ratios following gamma-irradiation (10, 20 and 30 Gy) represented as a function of time.



For cisplatin, a steep dose response curve was observed. Exposure to 0.1 µg/ml cisplatin (0.33 µM) over 4 hours induced a slightly reduced growth (P <0.05) on the first and second week although the outgrowth subsequently recovered. A concentration of 0.5 µg/ml (1.67 µM) cisplatin reduced the outgrowth capacity to about one fifth of the normal value (P <0.05), without recovery. After a 4 hour exposure to 1 µg/ml (3.33 µM) cisplatin, no outgrowth surface was formed surrounding the spheroids.

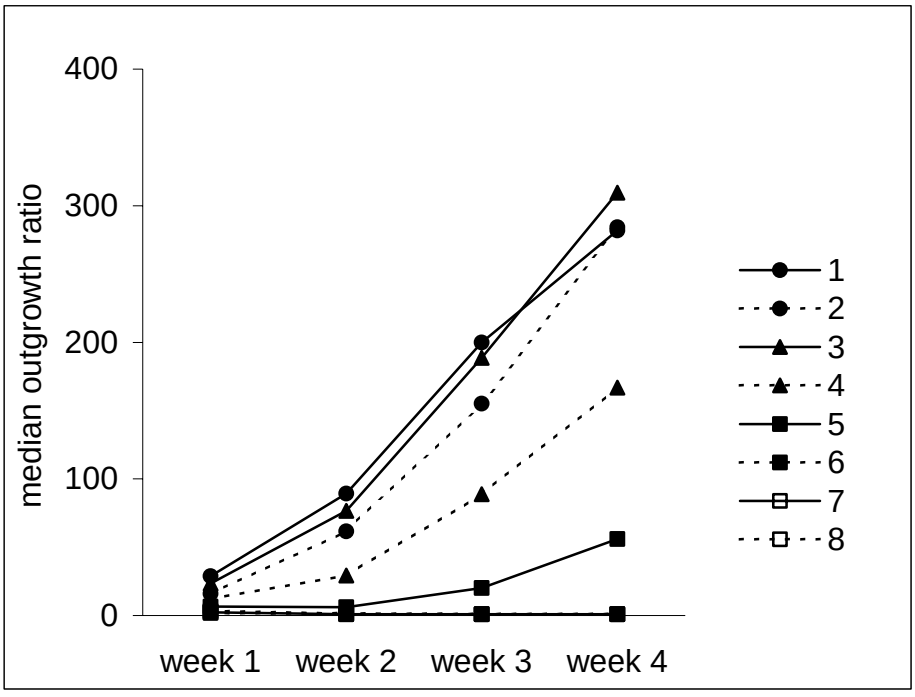
In the case of gemcitabin, concentrations of 3 ng/ml (10 nM) and 30 ng/ml (100 nM) were used during a 4 or 24 hour exposure. Only transient and minor effects were observed after a 4 or 24 hour exposure to 3 ng/ml gemcitabin. However, following a 4 hour exposure to 30 ng/ml gemcitabin the outgrowth was reduced significantly during the first three weeks, although the spheroids were able to recover at week 4. This was not the case for a prolonged exposure of 24 hours to 30 ng/ml of gemcitabine (P <0.05).

Spheroids exposed to 50 µg/ml 5-fluorouracil (0.38 mM) for 4 hours initially had a significant outgrowth reduction of 22 to 45% of the normal outgrowth (P <0.05) although recovery was seen at week 4. No major addition in toxicity was observed after doubling the concentration (0.77 mM) of 5-fluorouracil.

Screening for supra-additive effects of cytotoxic drugs and gamma-irradiation in HepG2-spheroids

The addition of concomitant irradiation (10 Gy) to the 0.1 µg/ml cisplatin exposure yielded a further reduction of the outgrowth potential on week 2. This was observed as a significant supra-additive effect (P <0.05). At later time points the outgrowth recovered partially to 44 and 59% at weeks 3 and 4 respectively (P >0.05). Combined with 0.5 µg/ml cisplatin, the irradiation inhibited the outgrowth completely (P <0.05). As a 4 hour exposure to 1 µg/ml cisplatin already impeded the formation of an outgrowth surface, no synergy could be demonstrated by adding 10 Gy (Figure 2).

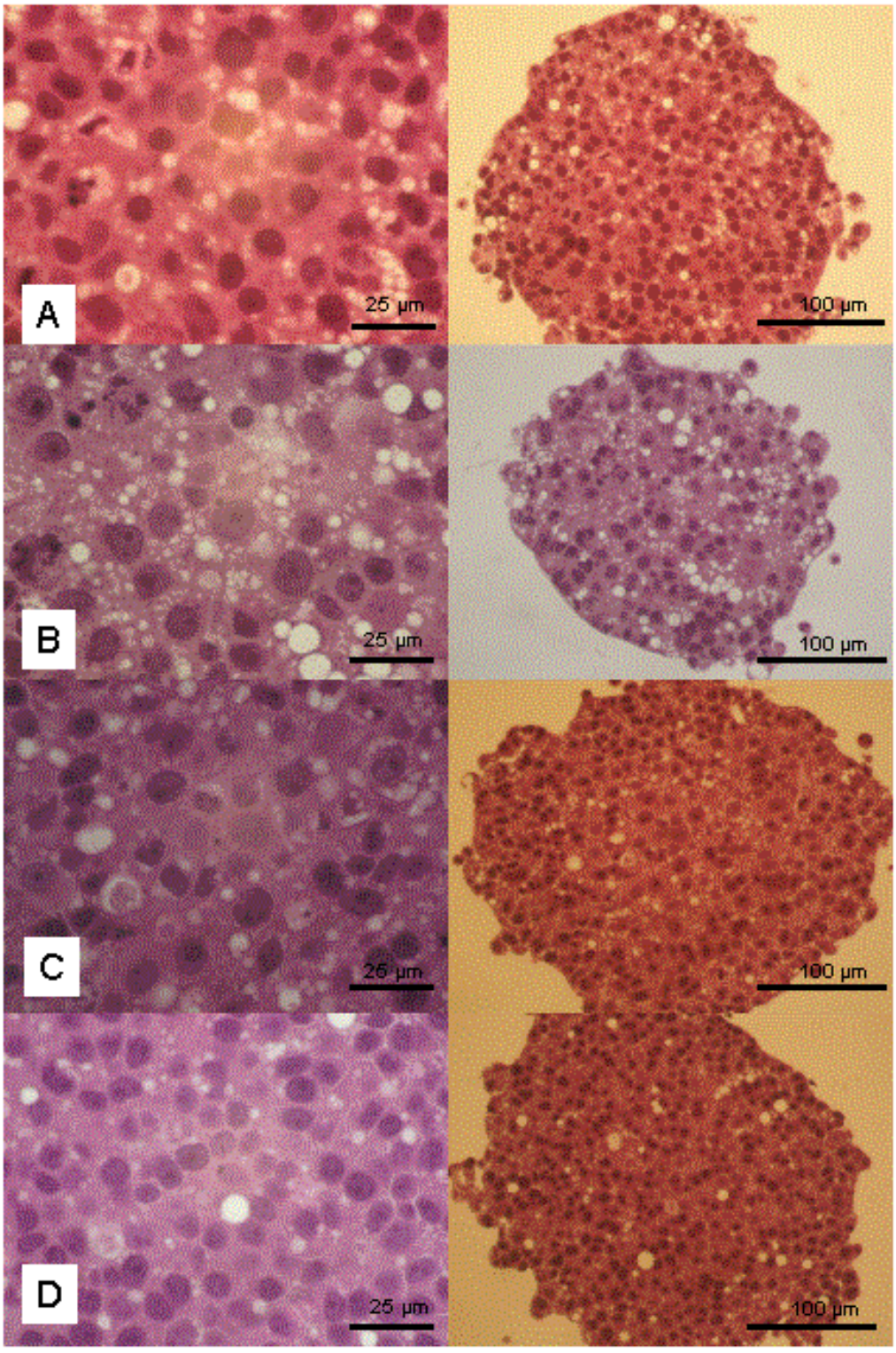
FIGURE 2: Median outgrowth ratios following a 4 hour exposure to cisplatin with or without concomitant irradiation. Control spheroids (1), 10 Gy (2), 0.1 µg/ml cisplatin (3), 0.1 µg/ml cisplatin and 10 Gy (4), 0.5 µg/ml cisplatin (5), 0.5 µg/ml cisplatin and 10 Gy (6), 1 µg/ml cisplatin (7), 1 µg/ml cisplatin and 10 Gy (8).



Hematoxyllin and eosin staining in histological samples of the spheroids confirmed the outgrowth assay: changes in the intercellular contacts were observed after exposure to 0.1 µg/ml of cisplatin with or without irradiation but higher concentrations induced necrosis and significant alterations in morphology. Two days after a 4 hour incubation with 1 µg/ml cisplatin, a large fraction of the cells became necrotic. As illustrated in Figure 3, the cell density was decreased in spheroids exposed to 0.5 µg/ ml cisplatin. Furthermore,

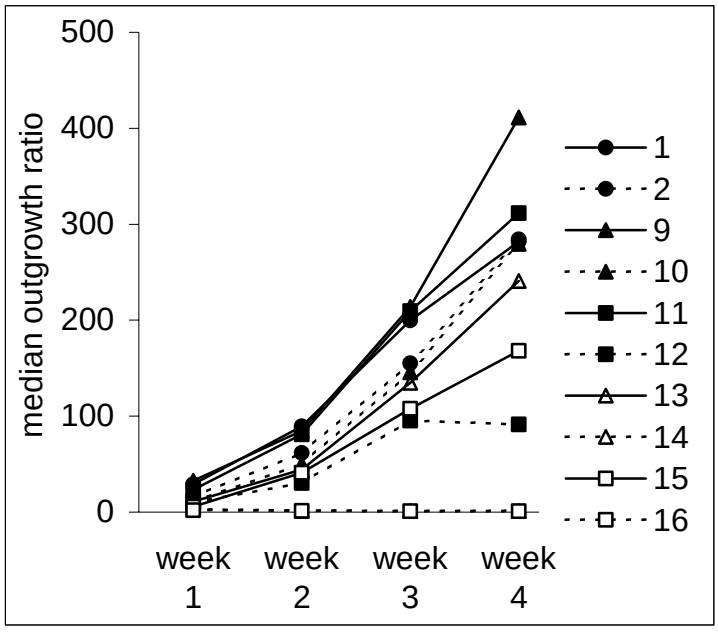
pycnotic nuclei, an increased vacuolization of the cells and a degradation of the intercellular contacts were observed. The addition of irradiation generated more pronounced changes.

FIGURE 3: Spheroids following various treatments. Control spheroids (A), irradiation with 10 Gy (B), exposure to 0.5 µg/ml cisplatin for 4 hours (C) and exposure to 0.5 µg/ml cisplatin for 4 hours with concomitant irradiation of 10 Gy (D).



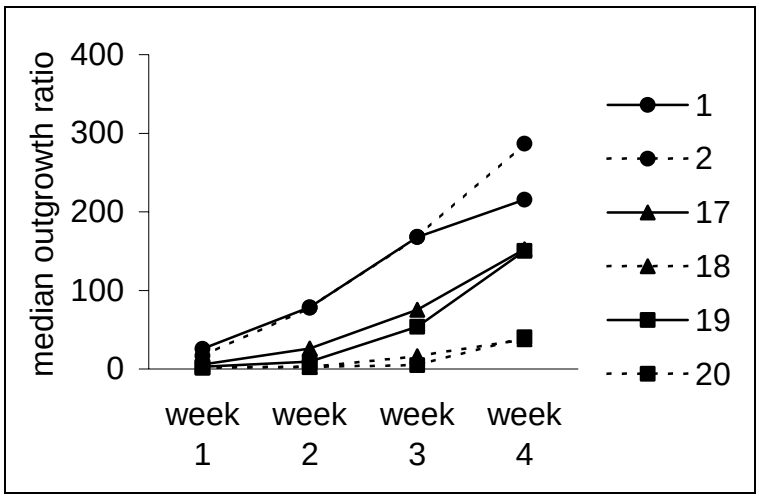
The addition of 10 Gy after a 4 hour incubation with gemcitabin (3 ng/ml) induced only a supra-additive effect for the first week (P <0.05). Recuperation occurred over the following weeks and was complete at week 4. However, a prolonged incubation time of 24 hours at the same low concentration reduced the outgrowth to about one third without any recuperation (P <0.05). When the concentration of gemcitabin was increased to 30 ng/ml, the irradiated spheroids lost their outgrowth potential completely. In this case only 10 % of the normal outgrowth occurred during the first week and less than 1 % of the normal outgrowth was seen at week 4. Moreover, in the case of exposure to 30 ng/ml gemcitabin, the incubation time did not affect the irradiated groups (Figure 4).

FIGURE 4: Exposure to gemcitabin for 4 or 24 hours with or without subsequent irradiation.
Median outgrowth ratios are represented as a function of time. The curves shown are for control spheroids (1), irradiation with 10 Gy (2), exposure to 3 ng/ml gemcitabin for 4h (9), 3 ng/ml gemcitabin for 4h and irradiation with 10 Gy (10), 3 ng/ml gemcitabin for 24h (11), 3 ng/ml gemcitabin for 24h and irradiation with 10 Gy (12), 30 ng/ml gemcitabin for 4h (13), 30 ng/ml gemcitabin for 4h and irradiation with 10 Gy (14), 30 ng/ml gemcitabin for 24h (15), 30 ng/ml gemcitabin for 24h and irradiation with 10 Gy (16).



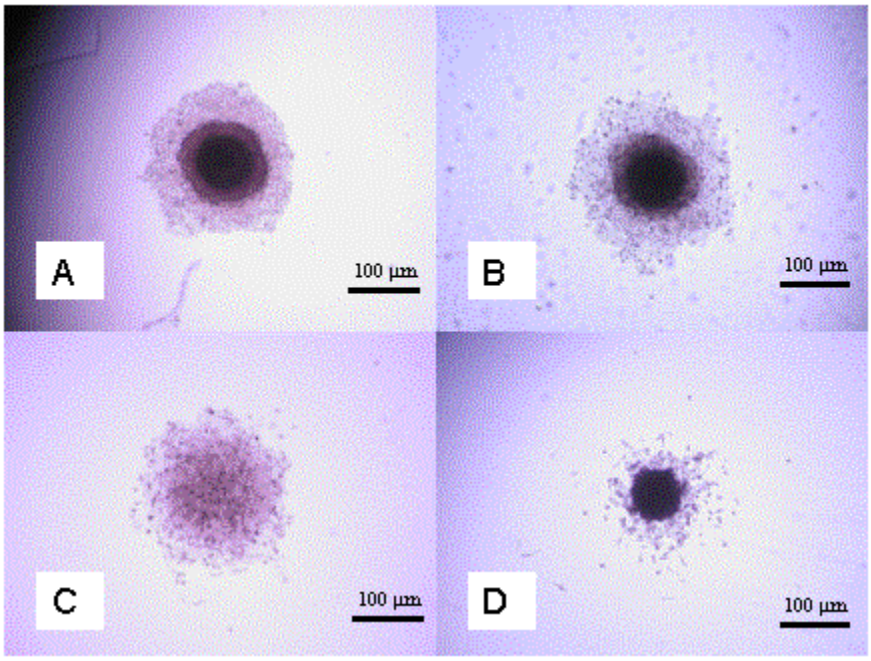
Irradiation of spheroids after incubation with 50 µg/ml 5-fluorouracil for 4 hours induced significant supra-additive effects, lasting until the fourth week. 71% of normal outgrowth was achieved in spheroids exposed to chemo alone whereas only 18% of normal outgrowth was observed if irradiation was added (P <0.05). Similar results are obtained with a concentration of 100 µg/ml (Figure 5).

FIGURE 5: Exposure to 5-fluorouracil (4 hours) with or without subsequent irradiation.
Median outgrowth ratios are represented as a function of time. Control spheroids (1), 10 Gy (2), 50 µg/ml 5-fluorouracil (17), 50 µg/ml 5-fluorouracil and 10 Gy subsequent (18), 100 µg/ml 5-fluorouracil (19), 100 µg/ml 5-fluorouracil and 10 Gy subsequent (20).



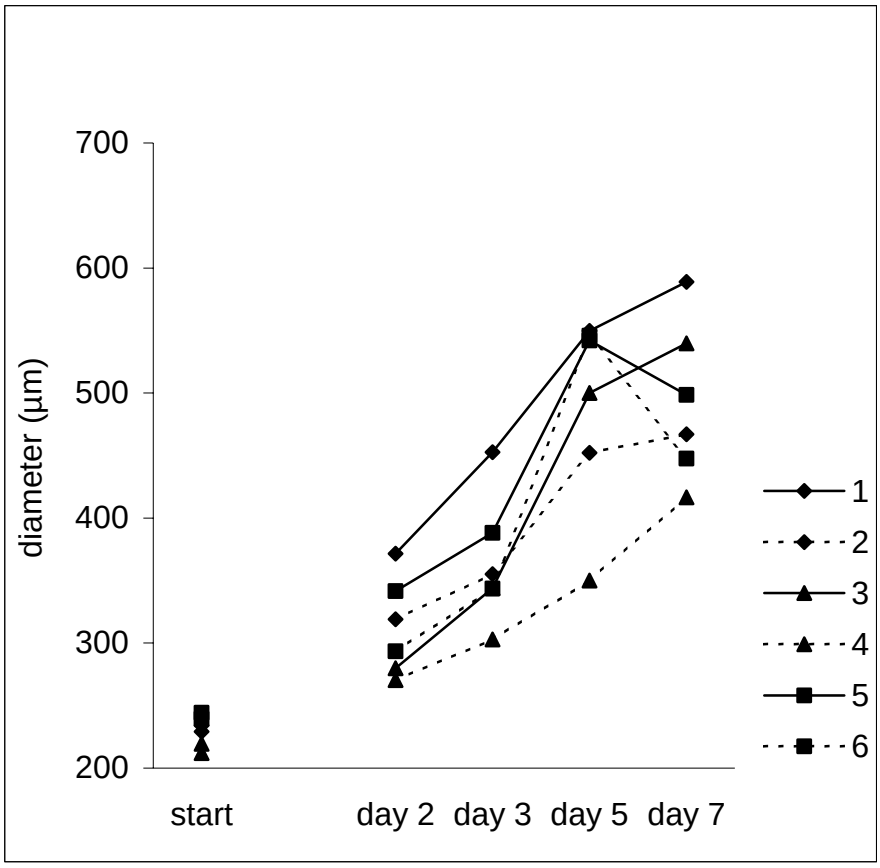
Spheroids with a reduced outgrowth capacity often expressed morphological changes. Qualitative changes after exposure to 100 µg/ml 5-fluorouracil such as loose cellular contacts, irregular architecture and a reduced density of the surrounding cellular surface are illustrated in Figure 6.

FIGURE 6: Microscopic view (10 x 20) one week after plating in a 24 well plate. Control spheroids (A), 10 Gy (B), 100 µg/ml 5-fluorouracil (4 hours) (C), 100 µg/ml 5-fluorouracil (4 hours) with subsequent irradiation of 10 Gy (D).



Three-dimensional growth results obtained within the first week after plating in an elisa-plate did not completely match the outgrowth assay. The irregular growth pattern is illustrated in Figure 7 for a series of spheroids exposed to 0.1 or 0.5 µg/ml cisplatin with or without concomitant irradiation.

FIGURE 7: The mean diameter of the spheroids, plated in an elisa-plate expressed as a function of time. Control spheroids (1), 10 Gy (2), 0.1 µg/ml cisplatin during 4 hours (3) and 0.1 µg/ml cisplatin for 4 hours with concomitant irradiation of 10 Gy (4), 0.5 µg/ml cisplatin for 4 hours (5) and 0.5 µg/ml cisplatin for 4 hours with concomitant irradiation of 10 Gy (6).



DISCUSSION

The three tested drugs are known radiosensitizers, and cisplatin and 5-fluorouracil are among the most effective agents in the treatment of hepatocellular carcinoma. However, objective response rates after systemic single-agent chemotherapy remain poor (*11*). In most instances response rates do not exceed 20% and a median survival of only 4 months is achieved (*12*). Gemcitabin has so far only been used for the treatment of HCC in phase II clinical trials, but its use in monotherapy is not recommended (*13*). More encouraging response rates of up to 50% were obtained after intra-arterial administration of single-agent chemotherapy (*14*). For sustained locoregional drug delivery the chemotherapeutic agent may be mixed in Lipiodol, a fatty acid

mixture retained in malignant hepatocellular cells. This treatment modality has yielded promising results (*15*), but the clinical use of so-called chemo-embolization is still under debate as considerable toxicity has been reported (*16*).

An alternative treatment modality consists of the intra-arterial administration of Lipiocis™ (Schering, Gif sur Yvette, France), Lipiodol labeled with Iodine-131 (*6,17*). In a randomized trial conducted by Raoul and coworkers Iodine-131 labeled Lipiodol and chemoembolization (70 mg cisplatin mixed in Lipiodol) appeared equally effective in terms of patient survival and tumor response, but tolerance to 131I-labeled Lipiodol was significantly better (*16*).
Although overall long-term survival remains poor, a combination of treatment modalities could result in improvement of outcome without induction of severe toxicity (*5*). Phase I clinical studies are being conducted to investigate the benefit of adding systemic cisplatin to locoregional irradiation by Lipiocis™ (*7*) and 5-fluorouracil and cisplatin were used with concurrent external beam irradiation (*18*). Of particular interest at present are Lipiodolized emulsions that remain in the tumor for long periods and release drugs in a sustained pattern. A wide variety of cytostatic drugs have been mixed successfully in Lipiodol which can also be radiolabeled with Iodine-131 or Rhenium-188, radionuclides that are routinely used in targeted radionuclide therapy (*19-21*).

The radiosensitizing capabilities of the tested agents are clearly demonstrated in the outgrowth assay for a 4 hour incubation with 0.5 µg/ml cisplatin, a 24 hour incubation with 3 ng/ml gemcitabine, a 4 hour incubation with 30 ng/ml and for 50 and 100 µg/ml exposure to 5-fluorouracil. For cisplatin and gemcitabin in particular, these doses are low compared to the safe plasma concentrations of these drugs in human use. The effect of 0.1 µg/ml cisplatin did not yield a sustained enhanced response to irradiation, whereas the effect of 1 µg/ml was too toxic to allow demonstration of an additive or supra-additive effect. In the case of gemcitabin, the incubation time played a crucial role in the outgrowth capacity. Similar observations have already been reported for other cell lines (*22*). In contrary to the two other drugs tested, there was no significant increase in toxicity after increasing the concentration of 5-fluorouracil as previously demonstrated for Hep G2 cells by Chenouffi et al (*23*).
The results of the three-dimensional growth curves are not completely consistent with the outgrowth assay. This can partly be explained by the poor prediction of normal growth patterns of multicellular tumor spheroids using traditional continuous growth models (*24*) and by the results of histological examinations, showing a reduced cell density in heavily exposed spheroids.
The follow-up period of one week is possibly too short to assess recovery from growth inhibition and longer observation periods are essential to discover significant differences between groups. However, extending the follow-up time of the three-dimensional growth experiments is not feasible because of the risk of fragmentation of control spheroids as they continue to grow. This is not the case in the outgrowth assay, in which a two-dimensional growth pattern is assessed. It was found that if the medium was refreshed weekly an excellent quality of the cultures could be maintained for 4 to 6 weeks.

Further research is warranted to evaluate the radiosensitizing capabilities of low doses of cisplatin and gemcitabin in combination with low dose rate irradiation, as is the case for radionuclide therapy with Iodine-131 labeled Lipiodol.

CONCLUSION

It is feasible to use HepG2- tumor spheroids as *in vitro* model for hepatocellular carcinoma to screen drugs in various combinations with irradiation. Analysis of the outgrowth ratios of spheroids cultured in 24-well plates for 4 weeks shows enhancement of the effect of gamma irradiation if pretreated with chemotherapy. The most toxic combinations with the 10 Gy gamma irradiation were found in exposures to 0.5 µg/ml cisplatin for 4 hours and 30 ng/ml gemcitabin for 4 or 24 hours.

REFERENCES

1. Monto A. and Wright TL. 2001. The Epidemiology and Prevention of Hepatocellular Carcinoma. *Sem. Oncol* 2001;28:441-449.
2. Schafer DF and Sorrell MD. Hepatocellular Carcinoma. *Lancet* 1999;353:1253-1257.
3. Kashef E, Roberts JP. Transplantation for Hepatocellular Carcinoma. *Semin Oncol* 2001;28:497-502.
4. Giovannini M, Elias D, Monges G, Raoul JL, Rougier P. Hepatocellular Carcinoma. *Br J Cancer* 2001;84:74-77.
5. Qian J, Feng GS and Vogl T. Combined interventional therapies of hepatocellular carcinoma. *World J Gastroenterol* 2003;9:1885-1891.
6. Novell JR and Hilson A.J. Iodine-131-Lipiodol for Hepatocellular Carcinoma: The Benefits of Targeting. *J Nucl Med* 1994;35:1318-1320.
7. Brans B, Van Laere K, Gemmel F, Defreyne L, Van Langenhove P, Troisi R, Van Vlierberghe H, Colle I, de Hemptinne B, Dierckx RA. Combining iodine-131 Lipiodol therapy with low dose cisplatin as a radiosensitizer: preliminary results in hepatocellular carcinoma. *Eur J Nucl Med Mol Imaging* 2002;29: 928-932.
8. Sutherland RM. Cell and Environment Interactions in Tumor Microregions. The Multicellular Spheroid Model. *Science* 1988;240:177-184.
9. Santini MT, Rainaldi G, Indovina PL. Multicellular tumour spheroids in radiation biology. *Int J Radiat Biol* 1999;75: 787-799.
10. Castañeda F and Kinne RK. Short exposure to millimolar concentrations of ethanol induces cell death in multicellular HepG2 spheroids. *J Cancer Res Clin Oncol* 2000;126:305-310.
11. Lin DY, Lin SM, Liaw YF. Non-surgical treatment of hepatocellular carcinoma *J Gastroenterol Hepatol* 1997;12: S 319-328
12. Leung TW and Johnson PJ. Systemic Therapy for Hepatocellular Carinoma. *Sem Oncol* 2001;28:514-520.
13. Ulrich-Pur H, Kornek GV, Fiebiger W, Schull B, Raderer M, Scheithauer W. Treatment of advanced hepatocellular carcinoma with bi-weekly high-dose gemcitabine. *Oncology* 2001;60:312-315.
14. Aguayo A. and Patt YZ. Non surgical treatment of hepatocellular carcinoma. *Sem Oncol* 2001;28:503-513.
15. Llovet JM, Real MI, Montana X, Planas R, Coll S, Aponte J, Ayuso C, Sala M, Muchart J and Bruix J. Arterial Embolisation or Chemo-embolisation versus Symptomatic Treatment in Patients with Unresectable Hepatocellular Carcinoma. A Randomised Controlled Trial. *Lancet* 2002;359:1734-1739.
16. Raoul JL, Guyader D, Bretagne JF, Heautot JF, Duveauferrier R, Bourguet P, Bekhechi D, Deugnier YM. and Gosselin, M. Prospective randomized trial of chemoembolization versus intra-arterial injection of 131-I-Iodized Oil in the treatment of Hepatocellular Carcinoma. *Hepatology* 1997;26:1156-1161.
17. Ho S, Lau WY, Leung TW and Johnson PJ. Internal radiation therapy for patients with primary or metastatic hepatic cancer. *Cancer* 1998;83:1894-1907.

18. Abrams RA, Pajak TE, Pajak TF, et al. Survival results among patients with alpha-fetoprotein-positive, unresectable hepatocellular carcinoma: analysis of three sequential treatments of the RTOG and Johns Hopkins oncology center. *Cancer Journal Am Sci* 1998;4:178-184.
19. Maeda H. SMANCS and polymer-conjugated macromolecular drugs: advantages in cancer chemotherapy. *Adv Drug Deliv Rev* 2001;46:169-185.
20. Yi SW, Kim YH, Kwon IC, Chung JW, Park JH., Choi YW and Jeong SY. Stable lipiodolized emulsions for hepatoma targeting and treatment by transcatheter arterial chemoembolisation. *J Control Release* 1998;50:135-143.
21. Yoon CJ, Chung JW, Park JH, Yoon YH, Lee JW, Jeong SY and Chung H. Transcatheter Arterial Chemoembolisation with paclitaxel-Lipiodol solution in rabbit VX liver tumor. *Radiology* 2003;229:126-131.
22. Lawrence TS, Eisbruch A and Shewach DS. Gemcitabine-Mediated Radiosensitization. *Sem Oncol* 1997;24: S7 24-28.
23. Chenouffi N, Raoul JL, Lescoat G, Brissot P and Bourguet P. In Vitro Demonstration of Synergy Between Radionuclide and Chemotherapy. *J Nucl Med* 1998;39:900-903.
24. Chignola R, Schenetti A, Chiesa E, Foroni R, Sartoris S, Brendolan A, Tridente G, Andrighetto G. and Liberati, D. Oscillating growth patterns of multicellular tumour spheroids. *Cell Prolif* 1999;32:39-48.

2.2. Assessment of supra-additive effects of cytotoxic drugs and low dose rate irradiation in an in vitro model for hepatocellular carcinoma

Assessment of supra-additive effects of cytotoxic drugs and low dose rate irradiation in an in vitro model for hepatocellular carcinoma

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ABSTRACT

Aim: The aim of this work was to screen cytotoxic drugs, relevant in the treatment of HCC, for enhancement of the effects of low dose rate (LDR) irradiation in an *in vitro* model for HCC. For comparison, all drugs were as well tested in combination with high dose rate (HDR) γ-irradiation. Methods: Multicellular tumor aggregates or "spheroids" of HepG2-cells were cultured and exposed to HDR or LDR irradiation by means of respectively external beam Cobalt-60 (^{60}Co) or Rhenium-188 (^{188}Re) dissolved in the culture medium for 24 h. Secondly, exposure to irradiation (10 Gy, by means of ^{60}Co or ^{188}Re) was combined concomitantly with a 24 h incubation with 5-fluorouracil (5 µg/ml), topotecan (0.1 µg/ml) or gemcitabine (30 ng/ml). Toxicity was evaluated by means of a quantitative spheroid outgrowth assay. Outgrowth for the various combinations was compared on each week till week 4 by means of Mann-Whitney statistical testing. Results: Absorbed doses of 10, 20 and 30 Gy following exposure to ^{188}Re, did not yield significant outgrowth differences compared to HDR irradiation exposures. For 5-fluorouracil, supra-additive effects lasting for 4 weeks were observed in combination with a HDR irradiation. For combination with ^{188}Re, the supra-additive toxicity was evident till week 3 but did not last till the fourth week. For topotecan and ^{188}Re, no supra-additive effects were seen whereas the addition of HDR irradiation at the end of the topotecan exposure yielded lasting supra-additive effects on the outgrowth. However, this was not the case if the HDR irradiation was performed at the start of the 24 h topotecan exposure. For gemcitabine, synergy with ^{188}Re was evident and lasted till week 4. Exposure to HDR irradiation at the end of the incubation with gemcitabine induced an even stronger synergic toxic effect on the spheroid outgrowth. No supra-additive effects were observed if HDR irradiation was added at the start of the incubation with gemcitabine. Conclusion: For all drugs tested, supra-additive effects were observed with HDR γ-irradiation if the timing of the irradiation was appropriate. For ^{188}Re, supra-additive effects were less pronounced and only evident with 5-fluorouracil and gemcitabine but not with concomitant exposure to topotecan.

INTRODUCTION

Hepatocellular Carcinoma (HCC) is the most frequently occurring malignant tumor in the liver. It is the fifth most common malignancy in men, and the eighth most common in women worldwide (*1*). Over 80% of patients presenting with HCC suffer from cirrhosis and the reduced liver reserve restricts treatment options in this patient group (*2*). A cure can be achieved through partial liver resection or transplantation, although the former is only feasible in less than 30% of patients and the latter is restricted by a shortage of donor organs (*3*). Patients presenting with HCC often have advanced disease with an underlying reduced liver function and thus a combination of treatment modalities might improve outcome and minimize toxicity in this vulnerable patient population (*4*). Radionuclide therapy by means of intra-arterial injection of radiolabeled Lipiodol, a fatty acid mixture labeled with Iodine-131 (^{131}I) or Rhenium-188 (^{188}Re) has yielded promising results (*5,6*). Phase I clinical studies are being conducted to investigate the benefit of adding chemotherapy to locoregional irradiation by means of radiolabeled Lipidol (*7,8*).
The objective of this study was to determine whether cytotoxic drugs used for the treatment of HCC could enhance the effect of low dose rate (LDR) irradiation *in vitro*. To reflect the *in vivo* situation, multicellular tumor aggregates or "spheroids" were cultured. Spheroids consist of different subregions, varying in the availability of oxygen, nutrients, pH, lactate and other substances. These factors influence the metabolic and proliferative status of the cells, resulting in a viable rim with proliferating cells and less mitotic activity in the deeper layers. Sutherland et al. reported that in general most of the proliferating cells are located in the outer three to five cell layers (75 µm) whilst quiescent cells are located more centrally. These cells often prove viable when removed from the deprived environment. Cells at a depth exceeding 160 µm may become necrotic (*9*). As spheroids consist of cell populations with variations in their degree of radio-resistance and cell cycle status, this model reflects the *in vivo* situation, such as micrometastasis or microregions within a vascularized tumor, more accurately than monolayers (*10*).

MATERIALS AND METHODS

A human cell line, HepG2, derived from a HCC was used to culture the spheroids (Bioreliance Ltd., Stirling, Scotland). After trypsinisation of a monolayer, spheroids were cultured by continuous gyratory shaking at 70 rpm (Heidolph Instruments, Schwabach, Germany) of a single HepG2-cell suspension in DMEM medium supplemented with 10% fetal bovine serum (Gibco, Invitrogen Corporation, Merelbeke, Belgium) and 5% CO2 at 37°C in PETG-flasks (Gosselin, Haezebrouck, France). After 4 to 10 days of shaking, the spheroids attained a mean (±SD) diameter of 205 ± 49 µm.
For exposure to LDR irradiation, the spheroids were incubated at 37°C with ^{188}Re (E β max 2.2 MeV, E γ 155 keV with an abundancy of 15%, physical half-life of 16.9 h) for 24 h. ^{188}Re was eluted from a ^{188}W/^{188}Re-generator, purchased from the Oak Ridge National Laboratory (Tennessee, USA), and the

IRE (Institut des Radio-Eléments, Fleurus, Belgium). For comparison, all experiments included a high dose rate (HDR) γ-irradiation by means of Cobalt 60 (^{60}Co) at a dose rate of 1 Gy per minute. Doses of 10, 20 and 30 Gy were delivered. Three cytotoxic drugs were used: 5 µg/ml 5-fluorouracil (Fluracedyl, Pharmachemie, Haarlem, The Netherlands), 0.1 µg/ml topotecan (Hycamtin, Glaxo-SmithKline, Middlesex, UK) and 30 ng/ml gemcitabine (Gemzar, Eli Lilly, Fegersheim, France). All cytotoxic drugs were incubated with the spheroids for 24 h. For combined modality experimental conditions, a fixed dose of 10 Gy was chosen. Irradiation was given concomitantly with the exposure to the cytotoxic drugs. For LDR irradiation, ^{188}Re was added to the culture medium in which the cytotoxic drug was dissolved and spheroids were washed after 24 h. Exposure to ^{60}Co was scheduled at 1 h following the start or 1 h before the end of the incubation with gemcitabine or topotecan. In case of 5-fluorouracil, ^{60}Co-irradiation was performed between 8 and 14 hours following the start of incubation with the cytotoxic drug.
The induced toxicity was evaluated by measuring the outgrowth potential of individual spheroids over 4 weeks (*11*). Within 24 h of treatment, individually selected spheroids were placed in 24 well plates coated for cell culture (Becton Dickinson Biosciences, Erembodegem, Belgium). The longest diameter and its perpendicular are measured using a calibrated ocular micrometer and light microscopy. After some days a monolayer, varying in surface area and density, is formed by motile and clonogenic cells that surround the original spheroid. The longest diameter of the confluent outgrowth surface and its perpendicular are measured at 7 day intervals for one month and the outgrowth ratio is calculated by dividing the average diameter by the average diameter measured on the day of plating. The outgrowth ratios of treated spheroids are compared with controls and those exposed to combined treatment to those treated by monotherapy using Mann-Whitney statistics (SPSS10.0-software package, Chicago, USA). The *P*-level of significance was set at 0.05. In the case of severe damage to the spheroid caused by the experimental conditions, no adherence or a reduced monolayer surface or so-called outgrowth surface was observed. An outgrowth ratio of 1 signifies a lack of adherence and a reduced outgrowth potential reflects diminished cell motility and clonogenicity (*12*). All series consisted of a minimum of 14 spheroids and all experiments were performed at least in duplicate.

RESULTS

Sensitivity of HepG2-spheroids to LDR versus HDR irradiation

Absorbed doses of 10, 20 and 30 Gy were delivered to the spheroids with ^{188}Re and ^{60}Co-irradiation. Independently from the applied dose rate, a dose of 10 Gy did not affect the outgrowth of the spheroids significantly compared to control spheroids (Figure 1). Exposure to 20 Gy and 30 Gy by means of ^{60}Co-irradiation yielded a significantly reduced outgrowth ratio compared to control spheroids of respectively 59% ± 47 SD and 4% ± 10 SD on week 4. For neither of the doses, a significant difference was observed in function of the applied dose rate. In Figure 2 the mean outgrowth ratios are represented over time.

FIGURE 1: Outgrowth surface showing complete recovery at week 4. Control spheroids (A), spheroids exposed to HDR irradiation (10 Gy) (B) and spheroids exposed to LDR irradiation (10 Gy) (C).

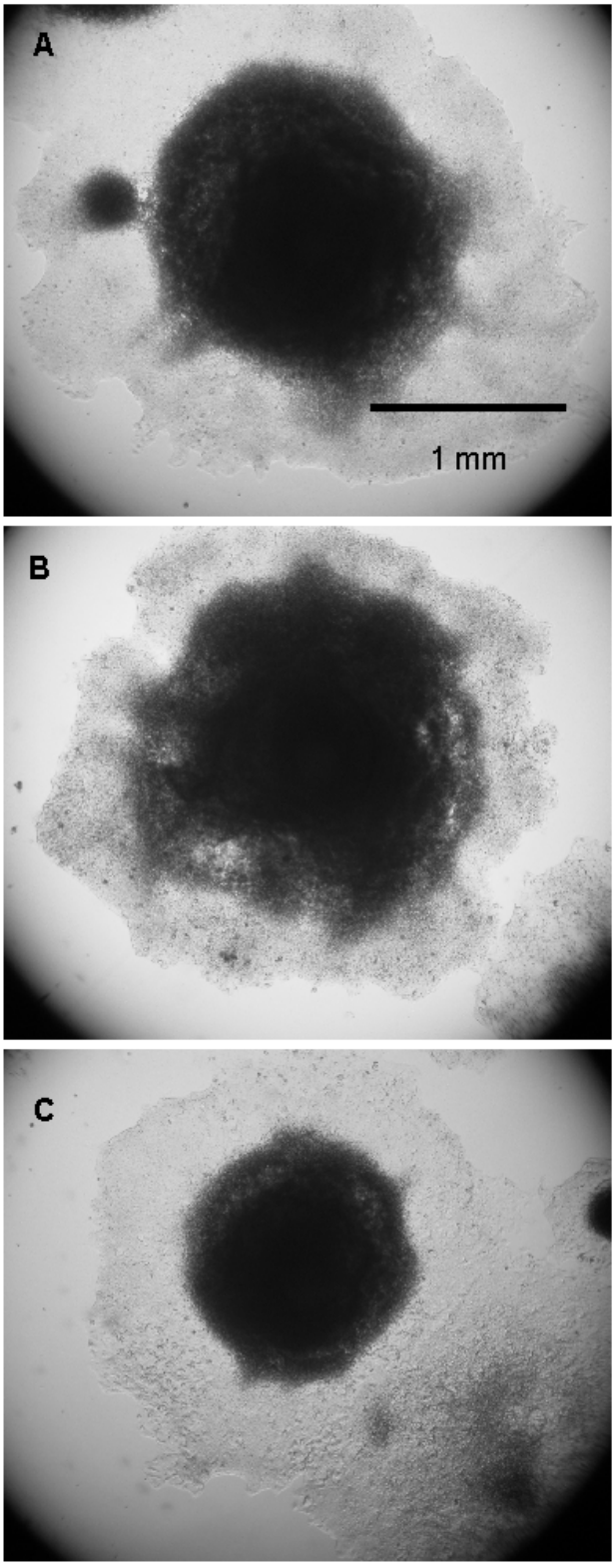
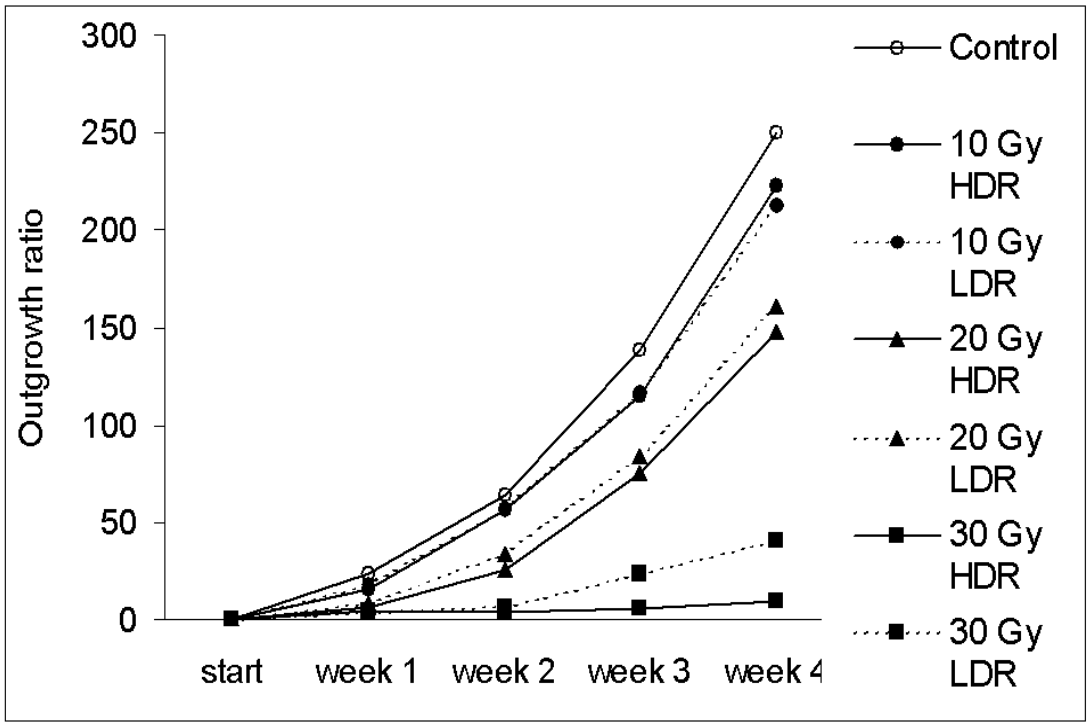


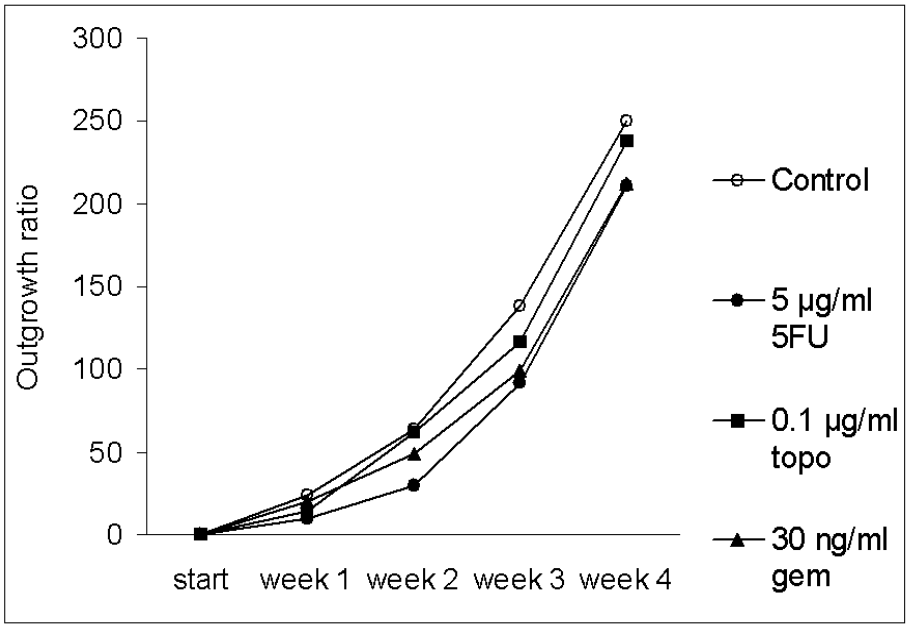
FIGURE 2: Mean outgrowth ratios following HDR- and LDR-irradiation (10, 20 and 30 Gy) represented as a function of time.



Sensitivity of HepG2-spheroids to the cytotoxic drugs

Spheroids exposed to 5 µg/ml 5-fluorouracil (38 µM) for 24 h had a small but statistically significant decreased outgrowth (85% ± 52 SD) at week 4. A similar observation was recorded for 30 ng/ml (100 nM) gemcitabine, whereas a full recovery of the outgrowth was seen following exposure to 0.1 µg/ml (0.22 µM) topotecan. The effects of the various drugs on the outgrowth ratios are depicted in Figure 3.

FIGURE 3: Mean outgrowth ratios following incubation for 24 h with 5 µg/ml 5-fluorouracil (5FU), 0.1 µg/ml topotecan (Topo) and 30 ng/ml gemcitabine (Gem) represented as a function of time.



Screening for supra-additive effects of cytotoxic drugs and LDR or HDR irradiation

Irradiation of spheroids by means of ^{60}Co whilst incubated with 5 µg/ml 5-fluorouracil for 24 h induced significant supra-additive effects, lasting until the fourth week. The first week the outgrowth was reduced to 10% (± 9 SD) of the outgrowth of control spheroids and on week 4 no recuperation had occurred (20% ± 24 SD). For concomitant exposure to 5-fluorouracil and ^{188}Re for 24 h, supra-additive effects were seen on week 1 (20% ± 19 SD) till week 3 (41% ± 26 SD) but on the fourth week no significant difference was observed in comparison to exposure to 5-fluorouracil alone. On all time points, the observed toxic effects were significantly stronger following the addition of HDR radiation to 5-fluorouracil compared to LDR radiation (Figure 4A).

Exposure of spheroids to 0.1 µg/ml topotecan for 24 h and concomitant HDR irradiation at the start of the incubation did not yield a significant decreased outgrowth compared to topotecan alone. However, if the ^{60}Co-exposure was scheduled at the end of the incubation with topotecan, a synergistic effect was seen. The first week the outgrowth was reduced to 19% ± 30 SD and at week 4 no recovery occurred (31% ± 30 SD). Adding ^{188}Re to the topotecan-exposure did not induce synergistic effects (Figure 4B).

The addition of 10 Gy HDR irradiation at the beginning of a 24 hr incubation with gemcitabin (30 ng/ml) induced a temporary additive effect on the outgrowth ratio. In the last 2 weeks, no statistical significant effect was observed in comparison to gemcitabine alone. If the exposure to ^{60}Co was planned at the end of the incubation with gemcitabine, a strong and lasting synergistic effect was evident (24% ± 27 SD at week 4). Similar observations,

although less pronounced (61% ± 48 SD at week 4), were obtained following the addition of LDR irradiation to the gemcitabine-exposure (Figure 4C).
In figure 5 a summary is given for all experimental conditions.

FIGURE 4 : Mean outgrowth ratios represented over time
A: following incubation for 24 h with 5 µg/ml 5-fluorouracil (5FU) with or without concomitant irradiation by means of ^{60}Co (10 Gy HDR, after 8-12 h of incubation with 5-fluorouracil) or ^{188}Re (10 Gy LDR). B: following incubation for 24 h with 0.1 µg/ml topotecan (Topo) with or without concomitant irradiation by means of ^{60}Co (10 Gy HDR, at the start or at the end of incubation with topotecan) or ^{188}Re (10 Gy LDR). C: following incubation for 24 h with 30 ng/ml gemcitabine (Gem) with or without concomitant irradiation by means of ^{60}Co (10 Gy HDR, at the start or at the end of incubation with gemcitabine) or ^{188}Re (10 Gy LDR).

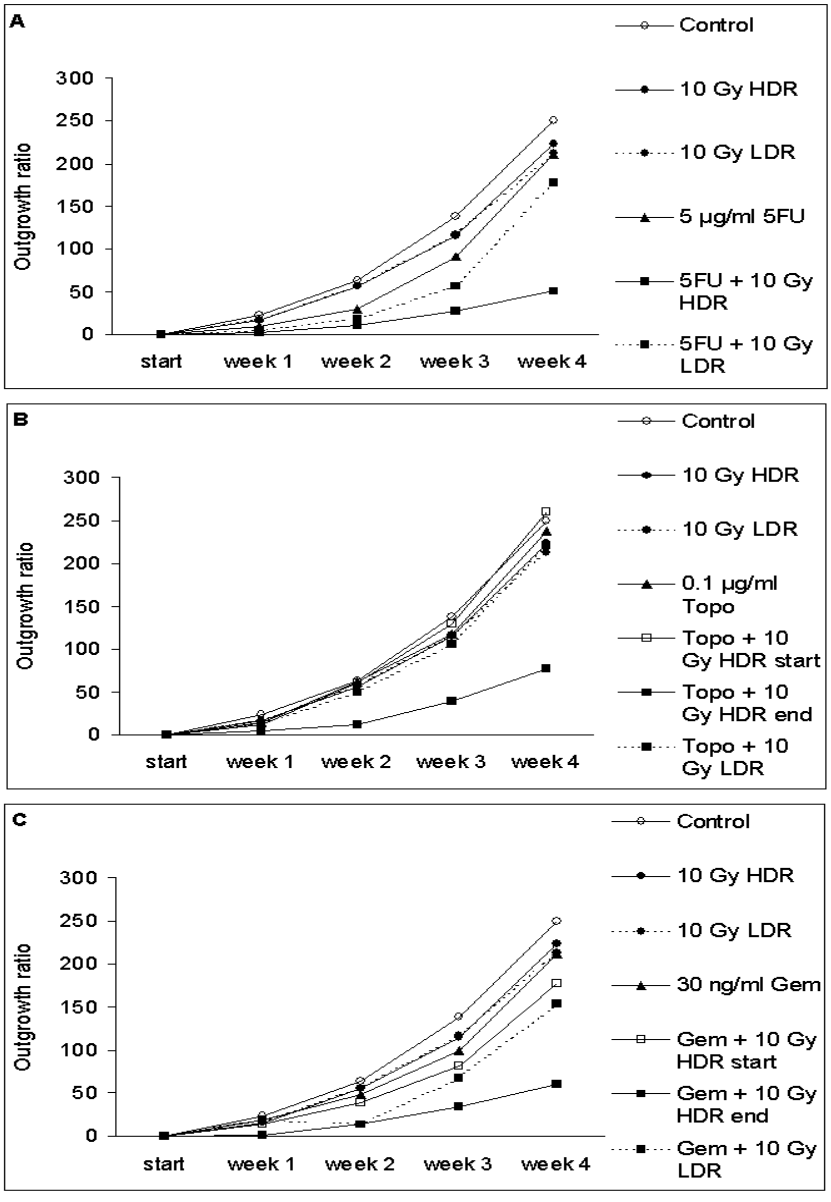
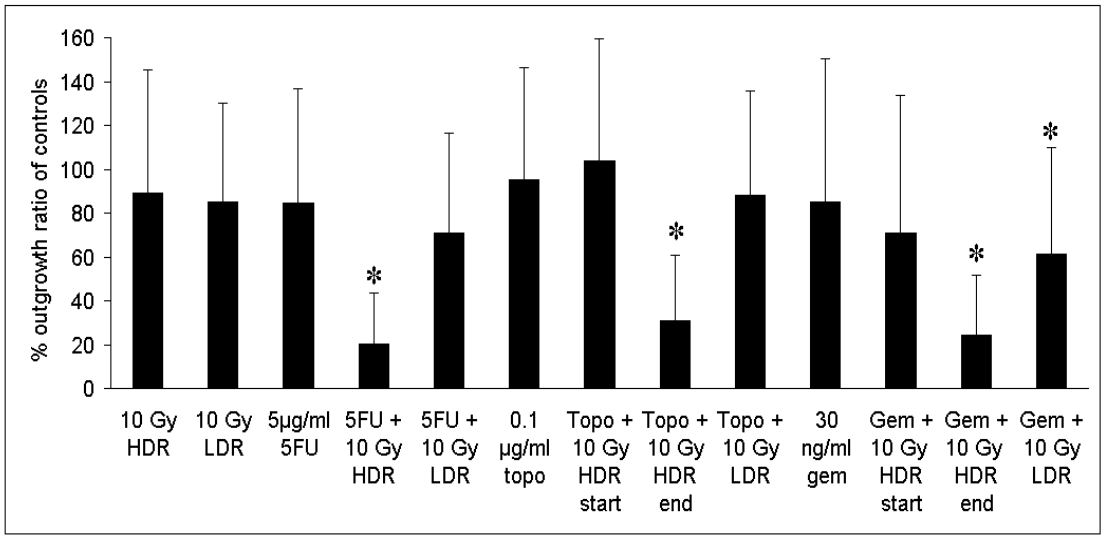


FIGURE 5: Relative outgrowth ratios at week 4 of spheroids following various treatments. All outgrowth ratios were normalized to the outgrowth of the control spheroids. Experimental conditions yielding significant (P <0.05) supra-additive effects lasting till week 4 are indicated (*).



DISCUSSION

The 3 tested drugs have been used in monotherapy for treatment of advanced HCC. However, objective response rates after systemic single-agent chemotherapy remain poor (*13-17*). More encouraging response rates of up to 50% were obtained after intra-arterial administration of single-agent chemotherapy (*18*). Chemotherapeutic agents may be mixed in Lipiodol, a fatty acid mixture retained in malignant hepatocellular cells, and injected in the hepatic artery with or without subsequent occlusion of the arterial supply of the tumor. This treatment modality has yielded promising results, but the clinical use of so-called chemo-embolization is still under debate and considerable toxicity has been reported (*19-21*). An alternative treatment modality consists of the intra-arterial administration of Lipiodol labeled with Iodine-131 (^{131}I) or ^{188}Re (*5,6,22*). In a randomized trial conducted by Raoul and coworkers ^{131}I labeled Lipiodol and chemo-embolization (70 mg cisplatin mixed in Lipiodol) appeared equally effective in terms of patient survival and tumor response, but tolerance to ^{131}I-labeled Lipiodol was significantly better (*21*). A combination of treatment modalities could result in improvement of outcome without induction of severe toxicity (*4*). Phase I clinical studies are being conducted to investigate the benefit of adding systemic cisplatin to locoregional irradiation by ^{131}I labeled Lipiodol and 5-fluorouracil and cisplatin were used with concurrent external beam irradiation (*7,23*). Of particular interest at present are lipiodolized emulsions that remain in the tumor for long periods and release drugs in a sustained pattern. A wide variety of cytostatic drugs have been mixed successfully in Lipiodol which can also be radiolabeled with ^{131}I or ^{188}Re (*24-26*).

In the first part of the present study a comparison was made between HDR and LDR irradiation by exposure of HepG2-spheroids to ^{60}Co (at a fixed dose rate of 1 Gy/minute) and ^{188}Re (at a mean dose rate of 6 mGy/minute over 24 h). In contrast to LDR irradiation, HDR exposure will deliver the absorbed dose before the cell can mobilize a significant reparative response (*27*). In accordance with the linear quadratic model employed in radiobiology, we expected that the biological effects of incubation with ^{188}Re would be less pronounced since intermittent repair of single strand DNA breaks was thought to occur. This would reduce the yield of double strand breaks induced by multiple DNA-hits (the quadratic term) (*28*). In our experiments a trend towards a stronger effect of HDR irradiation with increasing doses was found, although no statistically significant differences in outgrowth were detected between HDR and LDR irradiation for all absorbed doses tested (10 Gy up to 30 Gy). This finding suggests the relative overweight of the double strand DNA breaks induced by a single electron (linear term) in this experimental model. In a second part of our study, a number of drugs were screened for exhibiting synergistic effects when applied with HDR or LDR irradiation.

Enhancement of the effects of ionizing radiation by chemotherapy, is assumed to be related to (1) direct enhancement of the initial radiation damage by incorporating drugs into DNA, (2) inhibiting cellular repair, (3) accumulating cells in a radiosensitive phase or eliminating radioresistant phase cells, (4) eliminating hypoxic cells, (5) inhibiting the accelerated repopulation of tumor cells (*29*).
The tested drugs in the present study are known radiosensitizers. Gemcitabine and 5-flourouracil are pyrimidine analogues. Radiosensitizing effects are thought to be related to the perturbing effects on the deoxynucleotide metabolism. Nucleoside analogues act as DNA synthesis inhibitors and have the potential to inhibit the repair of genomic damage induced by ionizing radiation. Nucleoside analogues may also act as DNA chain terminators, and trigger an apoptotic response. Since DNA damage is induced in all phases of the cell cycle by radiation, this mechanism offers the prospect of extending the cytotoxicity of these analogues to non-S-phase cells.
Topotecan acts as an inhibitor of topoisomerase I, an enzyme essential for DNA replication, RNA transcription, chromosomal condensation and chromatid separation during mitosis (*30*). Topoisomerase I inhibitors are known to cause redistribution to the S-phase of the cell cycle.

The enhancement of the effects of ionizing radiation by these drugs depends on the applied drug concentration, the incubation time, the timing with the irradiation and the tested cell line (*31-33*). Animal studies with a murine hepatoma tumor model have shown the importance of administration of gemcitabine and 5-fluorouracil prior to HDR irradiation rather than post-irradiation. However, in this animal study supra-additive effects were only observed with gemcitabine and not with cisplatin, paclitaxel, adriamycin or 5-fluorouracil (*33*). Data concerning radiosensitizing effects of these cytostatic drugs combined with LDR irradiation are scarce and data focusing on HCC in

particular are lacking for gemcitabine. *In vitro* tests using a rat hepatoma cell line did not yield encouraging results for topotecan and ^{60}Co-irradiation. But in a rat model for HCC, increased therapeutic efficacy was described using a topoisomerase inhibitor dissolved in Lipiodol and ^{131}I based radioimmunotherapy (*34*). For 5-fluorouracil, no synergism with ^{131}I could be demonstrated in HepG2-monolayer experiments (*35*). In the present study, radiosensitizing capabilities are clearly demonstrated in the outgrowth assay following incubation with 5 µg/ml 5-fluorouracil, 30 ng/ml gemcitabine or 0.1 µg/ml topotecan and HDR irradiation if the timing between both modalities was appropriate. For LDR irradiation however, supra-additive effects that persisted until the fourth week following exposure to ^{188}Re were only evident with gemcitabine.

CONCLUSION

For palliative treatment of HCC, encouraging clinical data have been reported for locoregional chemotherapy mixed in Lipiodol as well as for ^{131}I or ^{188}Re labeled Lipiodol. The present study aimed to investigate whether the addition of 5-fluorouracil, gemcitabine or topotecan could enhance the anticancer effects of ^{188}Re labeled Lipiodol therapy in an *in vitro* model for HCC. A dose of 10 Gy did not affect the outgrowth of the spheroids significantly compared to control spheroids whereas doses of 20 Gy and 30 Gy yielded a significantly reduced outgrowth ratio. For neither of the doses, a significant difference was observed in function of the applied dose rate. Following the addition of 5-fluorouracil, gemcitabine or topotecan, supra-additive effects were observed with HDR γ-irradiation if the timing of the irradiation was appropriate. For ^{188}Re, supra-additive effects were less pronounced and only evident with 5-fluorouracil and gemcitabine but not with concomitant exposure to topotecan. Only in case of gemcitabine the supra-additive effects with LDR irradiation lasted till week 4.

REFERENCES

1. Monto A and Wright TL. The Epidemiology and prevention of hepatocellular carcinoma. *Sem Oncol* 2001;28:441-449.
2. Schafer DF and Sorrell MD. Hepatocellular carcinoma. *Lancet* 1999;353:1253-1257.
3. Kashef E and Roberts JP. Transplantation for hepatocellular carcinoma. *Semin Oncol* 2001;28:497-502.
4. Qian J, Feng GS and Vogl T. Combined interventional therapies of hepatocellular carcinoma. *World J Gastroenterol* 2003;9:1885-1891.
5. Sundram F, Chau TC, Onkhuudai P, Bernal P, Padhy AK. Preliminary results of transarterial rhenium-188 HDD Lipiodol in the treatment of inoperable primary hepatocellular carcinoma. *Eur J Nucl Med Mol Imaging* 2004;31:250-257.
6. Ho S, Lau WY, Leung TW. and Johnson PJ. Internal radiation therapy for patients with primary or metastatic hepatic cancer. *Cancer* 1998;83:1894-1907.
7. Brans B, Van Laere K, Gemmel F, et al. Combining iodine-131 Lipiodol therapy with low dose cisplatin as a radiosensitizer: preliminary results in hepatocellular carcinoma. *Eur J Nucl Med Mol Imaging* 2002;29:928-932.
8. Boschi A, Uccelli L, Duatti A, et al. A kit formulation for the preparation of 188Re-Lipiodol: preclinical studies and preliminary therapeutic evaluation in patients with unresectable hepatocellular carcinoma. *Nucl Med Commun* 2004;25:691-699.
9. Santini MT, Rainaldi G, Indovina PL. Multicellular tumour spheroids in radiation biology. *Int J Radiat Biol* 1999;75:787-799.
10. Sutherland RM. Cell and environment interactions in tumor microregions. The multicellular spheroid model. *Science* 1988;240:177-184.
11. Lambert B, De Ridder L, Slegers G, De Gelder V, Dierckx RA, Thierens H. Screening for supra-additive effects of cytotoxic drugs and gamma irradiation in an in vitro model for hepatocellular carcinoma. *Can J Physiol Pharmacol* 2004;82:146-152.
12. Castañeda F and Kinne RK. Short exposure to millimolar concentrations of ethanol induces cell death in multicellular HepG2 spheroids. *J Cancer Res Clin Oncol* 2000;126:305-310.
13. Leung TW and Johnson PJ. Systemic therapy for hepatocellular carinoma. *Sem Oncol* 2001;28:514-520.
14. Giovannini M, Elias D, Monges G, Raoul JL, Rougier P. Hepatocellular carcinoma. *Br J Cancer* 2001;84:74-77.
15. Ulrich-Pur H, Kornek GV, Fiebiger W, Schull B, Raderer M, Scheithauer W. Treatment of advanced hepatocellular carcinoma with bi-weekly high-dose gemcitabine. *Oncology* 2001;60:312-315.
16. Wall JG, Benedetti JK, O'Rourke MA, Natale RB, Macdonald JS. Phase II trial to topotecan in hepatocellular carcinoma: a Southwest Oncology Group study. *Invest New Drugs* 1997;15:257-260.
17. Yang TS, Lin YC, Chen YS, Wang HM, Wang CH. Phase II study of gemcitabine in patients with advanced hepatocellular carcinoma. *Cancer* 2000;89:750-756.

18. Aguayo A and Patt YZ. Non surgical treatment of hepatocellular carcinoma. *Sem Oncol* 2001;28:503-513.
19. Llovet JM, Real MI, Montana X, et al. Arterial embolisation or chemo-embolisation versus symptomatic treatment in patients with unresectable hepatocellular carcinoma. A Randomised Controlled Trial. *Lancet* 2002;359:1734-1739.
20. Reidy DL, Schwartz JD. Therapy for unresectable hepatocellular carcinoma: review of the randomized clinical trials-I: hepatic arterial embolization and embolization-based therapies in unresectable hepatocellular carcinoma. *Anticancer Drugs* 2004;15:427-37.
21. Raoul JL, Guyader D, Bretagne JF, et al. Prospective randomized trial of chemoembolization versus intra-arterial injection of 131-I-Iodized Oil in the treatment of Hepatocellular Carcinoma. *Hepatology* 1997;26:1156-1161.
22. Novell JR and Hilson AJ. Iodine-131-lipiodol for hepatocellular carcinoma: The benefits of targeting. *J Nucl Med* 1994;35:1318-1320.
23. Abrams RA, Pajak TE, Pajak TF, et al. Survival results among patients with alpha-fetoprotein-positive, unresectable hepatocellular carcinoma: analysis of three sequential treatments of the RTOG and Johns Hopkins oncology center. *Cancer Journal Am Sci* 1998;4:178-184.
24. Maeda H. SMANCS and polymer-conjugated macromolecular drugs: advantages in cancer chemotherapy. *Adv Drug Deliv Rev* 2001;46:169-185.
25. Yi SW, Kim YH, Kwon IC, et al. Stable lipiodolized emulsions for hepatoma targeting and treatment by transcatheter arterial chemoembolisation. *J Control Release* 1998;50:135-143.
26. Yoon CJ, Chung JW, Park JH, et al. Transcatheter arterial chemoembolisation with paclitaxel-lipiodol solution in rabbit VX liver Tumor. *Radiology* 2003;229:126-131.
27. Murtha AD. Radiobiology of Low-Dose-Rate radiation relevant to radioimmunotherapy. *Cancer Biother Radiopharm* 2000;15:7-14.
28. Hall EJ. Radiobiology for the Radiologist. Philadelphia: J.B. Lipincott Company, 1994.
29. Pawlik TM and K Keyomarsi. Role of cell cycle in mediating sensitivity to radiotherapy. *Int J Int J Radiat Oncol Biol Phys* 2004;59:928-942.
30. Chen Y, Okunieff P. Radiation and third-generation chemotherapy. *Hematol Oncol Clin North Am* 2004;18:55-80.
31. United Nations Scientific Committee on the Effects of Atomic Radiation. UNSCEAR 2000 Report. Combined effects of radiation and other agents. New York: United Nations, 2000.
32. Seong J, Kim SH, Suh CO. Enhancement of tumor radioresponse by combined chemotherapy in murine hepatocarcinoma. *J Gastroenterol Hepatol* 2001;16:883-889.
33. Lawrence TS, Eisbruch A and Shewach DS. Gemcitabine-mediated radiosensitization. *Sem Oncol* 1997;24:S724-728.
34. Roffler SR, Chan J and Yeh MY. Potentiation of radioimmunotherapy by inhibition of Topoisomerase I. *Cancer Res* 1994;54:1276-1285.

35. Chenouffi N, Raoul JL, Lescoat G, Brissot P and Bourguet P. In vitro demonstration of synergy between radionuclide and chemotherapy. *J Nucl Med* 1998;39:900-903.

Discussion

Discussion

Current status of locoregional radionuclide therapy for palliative treatment of HCC

A considerable number of patients suffering HCC present with advanced intrahepatic disease and thus curative treatments options such as partial liver resection or transplantation are not an option. Local ablative techniques such as PEI and RFA have yielded good long term results in retrospective series but are as well mainly applicable to patients with a limited number of lesions and tumoral diameters not exceeding 5 cm. Therefore, for many patients TACE remains as sole treatment modality that is widely available and for which evidence is available concerning its benefit in terms of patient survival (*1*). A meta-analysis conducted by Llovet and coworkers confirmed TACE in its role of standard treatment for HCC in this setting. However, several authors have pointed out that the conclusions of Llovet et al. are not that firm and mainly apply on well selected patients, suffering a Child-Pugh A degree of cirrhosis (*2,3*). In addition, a randomized study conducted by Raoul and coworkers showed a markedly better tolerance for ^{131}I-Lipiodol than for TACE with equal long term outcome and encouraged various research groups to continue the search for improved radionuclide treatment for HCC (*4*).
Optimization strategies consisted mainly of using radionuclides that are more 'user friendly' in terms of radioprotection and allow for administering higher activities, and thus aiming at delivering higher tumor doses. Attempts that have reached the stage of clinical trials consist mainly of ^{90}Y labeled microspheres and ^{188}Re-labeled Lipiodol. In the following section, the current status and future prospects of the various locoregional radionuclide therapies for HCC are discussed.

^{131}I-Lipiodol

Most published papers dealing with radionuclide therapy for HCC in a palliative setting concern the use of ^{131}I-Lipiodol, which is commercially available as Lipiocis™ (CIS Bio International, Gif sur Yvette, France) (*5-7*). Most reports using ^{131}I-Lipiodol agree on its good tolerance. Undesirable effects that are observed fairly frequently consist of moderate and temporary fever (29%), moderate and temporary disturbances of the biological liver tests (20%) and pain on injection (12.5%). Moderate and reversible leukopenia (7%) and serious diffuse infiltrative pneumopathies (2%) are observed more rarely. These diffuse infiltrative pneumopathies appear about 1 month after the injection of Lipiocis™ , most frequently after the second injection. They are clinically manifested by the appearance of dyspnea that is sometimes associated with dry cough and bilateral crepitations. The CT-scan shows infiltrates with the appearance of frosted glass, bilateral, surrounded by septal thickenings and sometimes evolving into fibrosis with some honeycomb-like images. This is a serious complication with a high mortality rate (Cis Bio International, personal communication).

Using either a drop on AFP levels or a reduction in tumor size as response criterion, reported response rates varied from 17 to 92% (*5-7*). Palliative treatment with ^{131}I-Lipiodol (n=65 patients) was compared to chemo-embolization (n=64 patients) in a prospective, randomized trial and survival after 6 months, 1, 2, 3 and 4 years were respectively 69%, 38%, 22%, 14% and 10% for the ^{131}I-Lipiodol arm versus 66%, 42%, 22%, 3% and 0% in the chemo-embolization arm (*4*). Importantly, treatment tolerance was much better in the ^{131}I-Lipiodol arm with only 3 reported serious side-effects versus 29 in the chemo-embolization arm and these results are in line with an earlier report by Bhattacharya et al. (*8*). While no survival benefit could be demonstrated for the whole group of palliative HCC patients, when focusing on HCC patients presenting with portal vein thrombosis a significant benefit in survival following ^{131}I-Lipiodol treatment versus tamoxifen, 5-fluorouracil or anti-inflammatory medication was however documented in a prospective randomized study by Raoul and coworkers (*9*). However, de Baere et al. did not confirm this benefit in patients with portal vein thrombosis in a non-controlled trial (*10*).

A dosimetry study was carried out at our institution in 15 patients who received a mean activity of 1.9 GBq (±0.2 SD) ^{131}I-Lipiodol. Patient dosimetry was performed based on 2 bi-planar total body scans using Monte Carlo simulation techniques. CT images of each patient were used to determine liver and tumor volume and position. Based on the data from the total body scans, the mean decay-corrected percentage of ^{131}I uptake in the total body at 7 days after administration was 72.8% (± 13.6 SD). At the same time the uptake in the normal liver, the tumor and the lungs was, respectively, 26.1% (± 8.3% SD), 17.2% (±7.5% SD) and 11.7% (± 5.4% SD). The percentage uptake in the total body two weeks following therapy had decreased to 53.1% (± 13.8% SD). The percentage uptake for the normal liver, the tumor and the lungs at the time of the second scan was 14.4% (± 4.3% SD), 14.5% (± 6.4% SD) and 7.4% (± 4.1% SD), respectively. The following mean absorbed doses were obtained: normal liver 7.8 Gy (range 4.0-11.8), tumor 129.5 Gy (range 68-246) and lungs 6.8 Gy (range 2.2-11.3). Eight patients received thyroid blocking by 100 mg potassium iodide daily and the absorbed dose to the thyroid was significantly lower for patients who received potassium iodide (7.2 Gy, range 3.4-10.7) than for the other patients (mean 13.8 Gy, range 8.0-21.9). For the total body dose, a mean value of 0.95 Gy (range 0.58-1.18) was obtained (*11*). Despite the good tolerance and the absorbed doses for the normal organs being far below the tolerance limits, an escalation in activity was not performed because the subsequent isolation of the patient for radioprotection concerns, would exceed 7 days and this is an important psychological drawback for the patient as well a significant extra cost.

Despite the numerous clinical reports, it remains unclear what the precise exclusion criteria for ^{131}I-Lipiodol treatment should be. Although never published as such, most centers exclude patients suffering an underlying cirrhosis with more than 7 points according to the Child-Pugh classification (*12*). According to Raoul et al. portal vein thrombosis does not imply unacceptable toxicity, whereas de Baere et al. do not recommend the use of ^{131}I-Lipiodol for

patients with portal vein thrombosis due to the occurrence of considerable toxicity in their experience (*9,10*). According to the recent guidelines provided by Cis Bio International, a radiography of the chest should be performed to document the baseline status in order to diagnose interstitial pneumopathy if symptoms would occur. However, maybe lung function testing should be added at baseline so that more discrete changes could be documented, allowing careful patient selection for the planning of repeated treatment sessions.

^{188}Re-labeled Lipiodol

Lee et al. developed a long-chain alkyl diaminedithiol derivative, abbreviated as ^{188}Re-HDD/Lipiodol, with satisfactory uptake and retention in an animal model (*13*). In a multi-center study sponsored by the International Atomic Energy Agency (IAEA) the safety and efficacy of this radioconjugate was assessed for treatment of inoperable HCC. Sixteen patients were treated in a pilot study with administered activities ranging from 1.8 GBq up to 7.7 GBq (*14*). The level of radioconjugate activity was based on radiation absorbed dose to critical organs, calculated following a 'scout-dose' of radioconjugate. The normal organs at greatest risk for toxicity were the normal liver and the lungs. In a subsequent study a further 54 patients were treated. The mean administered activity in this group was 4.6 GBq.

In this IAEA sponsored studies only a faint visualization of the kidneys was described, suggesting minimal renal elimination. However, the urinary excretion was not quantified (*14*). According to our measurements a mean of 44.1% of the administered ^{188}Re was excreted in the urine within 76 hours (*15*). ITLC analysis showed that the activity in the urinary samples was perrhenate. If the shorter physical half-life of ^{188}Re is taken into account, the value of 44.1% for ^{188}Re-HDD/Lipiodol compares favorably with the observations of Raoul et al. and Nakajo et al. using ^{131}I-Lipiodol (*16,17*). No visualization of the gastro-intestinal tract was described in the biodistribution studies using ^{131}I-Lipiodol nor in the initial reports on ^{188}Re-HDD/Lipiodol (*14,16-19*). In a minority of our patients faint and transient uptake in the small or large bowel was seen but fecal elimination was negligible compared to the renal excretion (*15*). In the animal study conducted by Lee et al. intestinal uptake was observed following intravenous administration of ^{188}Re labeled diaminedithiol derivatives in mice and the intestinal activity was thought to be result of bile excretion (*13*). In our study the uptake pattern and its transient character suggested biliary excretion rather than gastro-intestinal wall uptake.
Besides the liver, the lungs were the organs with considerable uptake in the majority of patients (*14,15,19*). We calculated a biologic half-life in the lungs for ^{188}Re of 8.7 (± 4.9 SD) days and this result is in line with earlier reports on the biologic half-life in the lungs following ^{131}I-Lipiodol therapy of 10.3 (± 5.2 SD) days (*11,15*). The relatively long biologic half-life in the lungs in both cases suggests that the activity in the lungs is present under a stable formulation from which diffusion to blood is minimal.

In the experience of Sundram et al. comprising about 70 patients, half of the patients reported mild and transient side effects, mainly consisting of low grade fever and right hypochondrial discomfort (*19*). In our experiences, ^{188}Re-HDD/Lipiodol is excellently tolerated in patients with well compensated cirrhosis. In this subset of patients, activities up to 7.0 GBq can be administered without the occurrence of unacceptable adverse events. For patients suffering underlying Child-Pugh B cirrhosis, the administration of 3.7 GBq in the arteria hepatica propria was accompanied by a considerable aggravation of pre-existing symptoms and laboratory aberrations. However, it remained unclear whether this was related to tumor progression rather than to the radionuclide therapy.
Sundram et al. adapted the administered activity of ^{188}Re-HDD/Lipiodol in function of a dosimetric analysis following the administration of a 'scout dose' of the radio-conjugate. The total administered activity was such that the liver, lung and bone marrow dose would not exceed respectively 30, 12 and 1.5 Gy. However, 2 cases of pleural effusion were reported by Sundram and coworkers and these were attributed to radiation induced pneumonitis (*19*). In our experience, 2 patients suffered prolonged coughing and dyspnea which exacerbated following the second treatment and required oral corticosteroid treatment. High resolution CT-scan of the lungs did not reveal stigmata of interstitial pneumopathy. The absorbed cumulative lung doses for both patients were estimated to be 10.4 and 11.5 Gy, hence too low to explain this evolution, although an increased sensitivity to ionizing radiation in these patients can not be excluded. An underlying allergy to components of the radio-conjugate could explain why exacerbation occurred in both cases following the second administration. Although it was not clear whether the occurrence of pulmonary symptoms was related to the ^{188}Re-HDD/Lipiodol therapy, we advocate that in the future, as is the case for ^{131}I-Lipiodol, particular attention is paid to patients developing pulmonary complaints.

According to the reports of the IAEA-trials, 13 patients had a partial response, 34 patients had stable disease and 23 patients suffered disease progression according to imaging. In our experience, 3 partial responses and 3 cases of progression were observed overall according to imaging whereas stable disease was recorded in the 46 remaining treatments. Unfortunately there were insufficient data about disease progression as measured by repeated imaging prior to treatment.
In the Western population, AFP-monitoring is only useful for the assessment of response in a limited number of patients since only 50% of patients present with a level of AFP that exceeds the critical limit of 400 ng/ml. Among our patients, 8 cases with rapidly increasing AFP prior to treatment were included. In 4 cases the tumor marker continued to rise 6 weeks later, in 2 cases it stabilized and in the 2 remaining patients the AFP was reduced by more than 90%.
Imaging by means of ultrasound, CT and MRI as well as by positron emission tomography (PET) using Fluoro-18 deoxyglucose (^{18}FDG) does not always reflect the true tumor load. Small lesions are easily missed and viability of tumor tissue is not well assessed (*20*). At our institution, 2 patients underwent

a liver transplantation which revealed over 99% of necrosis in their tumor although this had been reported as stable disease according to MRI. Further treatment of a larger numbers of patients is required to assess the therapeutic efficacy of ^{188}Re-HDD/Lipiodol and, in particular, long term outcome should be followed carefully rather than radiological response.

At present, the most important shortcoming of ^{188}Re-HDD/Lipiodol for treatment of HCC in patients with well compensated liver cirrhosis, consists of the labeling yield ranging between 50 and 70%. As pointed out in this work, the synthesis of activities exceeding 7.0 GBq routinely, poses a problem. A number of authors have focused on the development of new derivatives that may be produced at yields exceeding 95%. These include bis-(diethyldithiocarbamato) nitrido ^{188}Re Lipiodol (^{188}ReN-DEDC) and ^{188}Re-(S_2CPh)(S_3CPh)$_2$ Lipiodol (^{188}Re-SSS Lipiodol). While the ^{188}ReN-DEDC version was shown to be safe when given via the hepatic artery, either alone or in combination with TACE, in a series of 9 patients, data on ^{188}Re-SSS Lipiodol in HCC patients are currently lacking (*21,22*) .
For patients with Child-Pugh B cirrhosis, the administration of 3.7 GBq ^{188}Re-HDD/Lipiodol in the arteria hepatica propria did not yield encouraging results and possibly the treatment could be optimized by administering the radioconjugate as close to the tumor feeding arteries as possible.

Table 1: Summary of the 3 clinical studies, conducted in the framework of the present thesis, reporting the feasibility of the use of ^{188}Re-HDD/Lipiodol.

Chapter	**Patients**	**Therapies**	**Targeted administered activity**	**% Urinary excretion of administered activity** (mean ± SD)	**Response on MRI** (RECIST)	**AFP** (% reduction)
1.1.1.	11 (9 Child-Pugh A, 2 Child-Pugh B)	16	3.7 GBq	44.1 ± 11.7% over 76 h	PR: 1 SD: 11 PD: 1	5/8 ↓ (19-90%)
1.1.2.	28 (Child-Pugh A only)	35	4.8-7.0 GBq	41.7 ± 9.7% over 46 h	PR: 1 SD: 28 PD: 2	8/17 ↓ (19-97%)
1.1.3.	12 (Child-Pugh B only)	12	3.7 GBq	36.2 ± 5.7% over 52 h	PR: 1 SD: 7 PD: 0	3/9 ↓ (23-63%)

Radiolabeled microspheres

HCC are rich in vasculature and almost exclusively dependent on arterial blood supply while normal liver tissue receives most of its flow from the portal vein. Based on this difference in blood supply between HCC and normal liver tissue, microspheres with a diameter between 20 and 50 μm when injected into the hepatic artery, will selectively lodge in the vascular bed of HCC.

^{90}Y labeled microspheres

Early experiences with ^{90}Y labeled ceramic microspheres yielded disappointing results due to leaching of ^{90}Y from the microspheres, leading to bone marrow toxicity. In the 1980s a 25 μm glass microsphere (Therasphere™, MDS Nordion, Canada) and a 35 μm resin-based ^{90}Y labeled microsphere (Sirtex™, Sirtex Medical Ltd, Australia) were developed and these vehicles proved stable (*5*). The initial experiences with both types yielded promising results and it was proven feasible to deliver high liver (including tumor) doses (50 up to 150 Gy) (*5,23-27*). In all recent studies the therapeutic approach consists of treating the affected liver lobe if the patient presents with unilobar disease. Patients with bilobar disease receive whole-liver treatment spread over 2 treatment sessions. For estimating the required activity for Therasphere™ treatment, the liver volume planned to treat is determined by CT or MRI, and an algorithm is used. Before treatment, a percutaneous catheter is inserted into the femoral artery for angiography and selective visceral catheterization and a ^{99m}Tc-macroaggregated albumin (MAA) scan is performed to test for any gastrointestinal flow and to estimate the percentage of injected activity that might be shunted to the lungs. Lung doses exceeding 30 Gy following a single treatment or a cumulative dose exceeding 50 Gy should be avoided in order to prevent radiation pneumonitis (*26*). Although high activities are administered, no clinical significant bone marrow toxicity is observed. Severe toxicities mainly consist of hepatic failure and in rare occasions radiation pneumonitis, gastro-intestinal bleeding or cholecystitis (*25*).

It was shown by Goin et al. that 90 day mortality following Therasphere™ treatment could substantially be reduced by screening candidates for the presence of a number of risk factors such as infiltrative growth pattern, bulk disease, AST or ALT >5xULN, more than half of the liver volume replaced by tumor and albumin <3g/dL, bilirubin ≥2mg/dL (*28*). In low risk patients doses to the liver (including the tumor) as high as 150 Gy on a single administration and as high as 268 Gy on repeated administrations were well tolerated (*29*). The application of high activities (up to 13 GBq cumulatively) of ^{90}Y-labeled resin microspheres was also shown to be feasible in patients suffering HCC (*5,30*). Following the occurrence of a limited number of cases with radiation pneumonitis, the use of a pre-treatment ^{99m}Tc-MAA scan was implemented and patients with lung shunting exceeding 13% should not be treated according to the experiences of Leung and coworkers (*31*). In a phase I/II study by the same group tumor regression studied in 18 inoperable HCC patients was found to be dose-related. Progression or static disease occurred in a higher proportion

of patients whose tumor received <120 Gy. Also, in these patients survival was much poorer when compared to those whose tumor received >120 Gy (26.2 weeks versus 55.9 weeks) (*27*). In an attempt to more accurately predict tumor response and lung toxicity, a simple mathematical model was developed using the MIRD-formulation based on ^{99m}Tc-MAA scans. Using a beta-probe at laparotomy the validity of the ^{99m}Tc-MAA scan-derived estimated doses was evaluated both in a pre-clinical and clinical setting (*30*).

For both types of microspheres promising radiological response rates (27-38%) were claimed, but methodological differences make it impossible to compare results. A few cases of complete responses have been reported and survival compared favorably with historical controls (*5,23,27*).
In contrast to Therasphere™, no safety data are available concerning the use of Sirtex™ in patients with impeded venous flow but considering the embolizing effects of Sirtex ™ its use should be avoided in patients with portal vein thrombosis whereas Therasphere™ might have a role to play in this particular setting (*32*).

In contrast to ^{131}I, ^{90}Y lacks the emission of gamma-rays and thus treatment with ^{90}Y-labeled microspheres is feasible on an outpatient or 24 hour hospitalization basis (*32*). However, patients should be admitted twice to the hospital, since a prior angiography and ^{99m}Tc-MAA scan are recommended to avoid lung toxicity. If both lobes should be treated, in total 3 interventions should be performed. In addition, the cost price of ^{90}Y labeled microspheres for a single administration to date is about 6 times higher than for Lipiocis™.

^{32}P labeled microspheres

Besides ^{90}Y labeled microspheres, some clinical experience was gained with ^{32}P glass microspheres (*33*). In a comparative study, including 111 unresectable HCC patients, Liu et al. compared the therapeutic effect of intra-arterial ^{32}P glass microspheres (n=30 patients), to intra-arterial ^{32}P glass microspheres combined with half a dose of TACE (adriamycine 30 mg/m² + cis-diammine dichloroplatinum + iodized oil, n= 49 patients) and TACE alone (n=18). Results obtained using intra-arterial ^{32}P glass microspheres closely approximated those obtained using TACE alone. In contrast, results obtained using intra-arterial ^{32}P glass microspheres combined with half a dose of TACE were significantly better than those obtained using TACE alone.

$^{186/188}$Re labeled microspheres

Wunderlich et al. developed an efficient method of radiolabeling biodegradable human serum albumin (HSA) microspheres with ^{188}Re using a simple kit. One advantage of HSA microspheres compared to other labeled particles (e.g. human macro-albumin) is the uniformity of their size with a mean particle diameter of about 25 µm. Another advantage is the biocompatibility and *in vivo* degradability. The occlusion of blood vessels following intra-arterial application is only temporary (*34*). Clinical data on its use in patients suffering colorectal

liver metastasis suggest a good *in vivo* stability. As reported for ^{90}Y-labeled microspheres and in contrast to our experiences with radiolabeled Lipiodol, only minimal radioactivity is retrieved in the urine (*35*).
For ^{186}Re and ^{188}Re glass microspheres and ^{166}Ho-AcAc PLA-microspheres a therapeutic effect in animal models was shown but clinical data so far are lacking (*36-38*).

Radiolabeled microspheres versus Lipiodol

In Table 2 the advantages and disadvantages of the use of various types of radiolabeled microspheres are summarized and compared with radiolabeled Lipiodol.

TABLE 2: Comparison of the the various types of radiolabeled microspheres and Lipiodol derivatives.
* according to Belgian guidelines. ** depends on the number of patients treated per purchased ^{188}W/^{188}Re-generator.

	^{90}Y-labeled glass microspheres *Therasphere ™*	^{90}Y-labeled resin microsphere *Sirtex ™*	^{188}Re-labeled HSA microspheres	^{188}Re-HDD/ Lipiodol	^{131}I-Lipiodol *Lipiocis ™*
In vivo stability	excellent	excellent	good (preliminary data)	moderate	moderate
Need for hospitalization *	day clinic	day clinic	not defined: 24-48h?	24-48h	5-7 days
Applicable in case of portal vein thrombosis	yes	no	no data available	yes	yes
Visual control during and following administration by means of X-rays	limited	limited	limited	excellent	excellent
Visual control following administration by means of scintigraphy	yes	yes	yes	yes	yes
Commercially available in European Community	no	yes	no	no	yes
Cost of radiopharmaceutical per treatment	8.000-12.000$	8.000-12.000 $	Low production cost**	Low production cost**	1.500-2.000$
Clinical reports on treatment of HCC available	++ uncontrolled trials	+ uncontrolled trials	-	+ uncontrolled trials	+++ including randomized controlled trials

Locoregional radionuclide therapy as part of a multimodality approach

Surgery is the mainstay of curative treatment. No firm evidence for improved long term outcome to date is available for any adjuvant or neoadjuvant treatment strategy (*39*). Some evidence was established for the use of polyprenoic acid and ^{131}I-Lipiodol following partial liver resection in prospective randomized trials (39,*40*).

Locoregional radionuclide therapy as bridging strategy to transplantation

Neo-adjuvant treatment in patients meeting transplant criteria aims at preventing patients to drop out from the waiting list or to prevent disease progression while a donor is sought for living organ donation. Results of neo-adjuvant treatment strategies should be evaluated in terms of long term outcome, based on the number of patients intended to transplant. It is known that malignant cells have often spread to distant sites at the moment of transplantation and subsequent proliferation is enhanced by the immunosuppressed status of the patients post-transplantation. As a consequence, a multimodality approach combining local, locoregional and systemic treatment options seems to be essential in preventing recurrent disease.

The locoregional component of the neo-adjuvant treatment should target the entire liver since the presence of more than 3 tumors is considered as a contra-indication for transplantation is many centers. In that view intra-arterial radionuclide therapy or chemotherapy are appealing approaches. However the use of ^{131}I-Lipiodol excludes patients from the waiting list for 6 weeks due to radioprotection issues. In case of ^{188}Re, removal from the waiting list is recommended for only for one week due to its 17 hour physical half-life. This benefit is less pronounced for radionuclides with an intermediate physical half-life such as ^{90}Y (2.7 days).

Besides eventual therapeutic benefits, radiolabeled Lipiodol treatment might have additional diagnostic value in the work-out of potential transplant candidates. Before administering the radioconjugate, a diagnostic hepatogram including an indirect mesentericoportography was performed, which has proven of important added value for the surgeons in some cases.

Radionuclide therapy combined with cytostatic drugs

A combination of treatment modalities could result in improvement of outcome without induction of severe toxicity. Phase I clinical studies are being conducted to investigate the benefit of adding chemotherapy to locoregional irradiation by ^{131}I, ^{188}Re or ^{32}P based radionuclide therapy and 5-fluorouracil and cisplatin were used with concurrent external beam irradiation (*21*,*33,42,43*). Of particular interest at present are Lipiodolized emulsions that remain in the tumor for long periods and release drugs in a sustained pattern. A wide variety of cytostatic drugs have been mixed successfully in Lipiodol which can also be radiolabeled with ^{131}I or ^{188}Re (*44-46*).

The tested drugs in the presented work are known radiosensitizers and the radiosensitizing capabilities were clearly demonstrated in the outgrowth assays with high dose rate irradiation. Data concerning radiosensitizing effects of cytostatic drugs combined with low dose rate irradiation are scarce in the literature and thus we focused at combined effects of cytostatic drugs and ^{188}Re (low dose rate irradiation) in the second part of this thesis (*47*). With increasing doses, there was a trend towards stronger anti-tumoral effects if high dose rate irradiation was applied compared to low dose rate. It was expected that an absorbed dose delivered at low dose rate would not be as effective as if delivered at high dose rate. However, no statistically significant differences in therapeutic effect between high dose rate alone and low dose rate alone were observed. This was in contrast with the combined modality experiments, showing clearly that supra-additive effects of ^{188}Re and cytostatic drugs were less pronounced than for combinations with external beam ^{60}Co (high dose rate) irradiation.

Future prospects for ^{188}Re-based radionuclide therapies

The use of an on-site ^{188}W/^{188}Re-generator provides a good yield of carrier-free ^{188}Re routinely for radionuclide therapy (*48*). The main advantages of ^{188}Re are the relatively short physical half-life, the high energy of the β-particle emission and the γ-emission of 155 keV (15%) that allows imaging. The use of ^{188}Re for radionuclide therapy generates less concerns over radiation protection compared to ^{131}I. In addition, an on-site generator enables hospitals in remote areas or in regions with limited transport facilities to produce their own radiopharmaceuticals. This is particularly useful for less developed countries with a high prevalence of HCC since other commonly used therapeutic radiopharmaceuticals require a nuclear reactor for its production (^{131}I, ^{90}Y).

Most publications so far have focused on the use of ^{188}Re for bone pain palliation (*49-51*). Although encouraging results were reported, various aspects of this treatment modality remain unclear. Some authors prefer the use of low β-energy emitters for treatment of painful bone metastasis, because it is assumed that such radionuclides would exhibit less bone marrow toxicity. However, little evidence for such a strategy is available. ^{188}Re-HEDP and ^{153}Sm-EDTMP, show similar pain palliation effects in patients suffering from breast and prostate cancer. There were no differences in bone marrow toxicity reported between the higher β-energy ^{188}Re-HEDP and the lower β-energy ^{153}Sm-EDTMP in a comparative study (*52*). Other authors suggest enhanced antitumoral effects if high energy β-emitters are used and propose aggressive treatment in an earlier disease stage in stead of using these radiopharmaceuticals only in end-stage patients suffering intractable bone pain. The electrons emitted by ^{188}Re have a range of 3 to 5 mm in osseous tissue and thus target tumor as well as surrounding bone trabeculae and periostium. This would lead to cytotoxic effects in the outer layers of the tumor and repeated treatment with short intervals would prevent new tumor growth and eradication of deeper tumor layers (*53*). Another approach consists of including other treatment modalities such as autologous stem cell rescue and chemotherapy to a radionuclide treatment scheme (*54*). Future research should focus on the potential antitumoral effects of such aggressive treatment strategies and their impact on quality of life as well as survival.
So far, no studies with ^{188}Re-HEDP were carried out in patients with compromised bone marrow or impaired renal function. However, in daily practice, a considerable number of patients presenting with bone pain would not meet the inclusion criteria of the published phase I studies. In addition, the efficacy of radionuclide therapy for bone metastasis of other malignancies than prostate and breast cancer should be investigated.

Radioimmunotherapy for hematological malignancies is also an application where clinical results so far are very promising. However, it remains unclear what role ^{188}Re will play in radioimmunotherapy for solid tumors. The use of high molecular weight IgG could have a negative impact on the tumoral uptake kinetics of the radiopharmaceutical. In the case of slower tumoral uptake, the physical half-life of ^{188}Re of 16.9 hours would be relatively too short. On the

other hand the use of antibody fragments bears the risk of radiation nephritis. It remains to be elucidated if cationic amino acid infusions are able to prevent excess renal uptake of the radiolabel in such a setting.
Although various animal experiments on the subject were published several years ago, no recent clinical advances using ^{188}Re-labeled somatostatin analogues or other peptides were reported (*55-58*).

For treatment of liver tumors, important preclinical and clinical progresses were made in the last five years. Attempts to use ^{188}Re for treating HCC as well as liver metastasis, consist of a number of radiolabeled Lipiodol based radiopharmaceuticals (*13-15,19,21,22,59*) as well as radiolabeled glass, polylactic acid and albumin microspheres (*34-36*). At present most clinical data are derived from phase I studies using ^{188}Re-HDD/Lipiodol for palliative treatment of HCC. In the present work it was shown that patients with well compensated liver dysfunction, have an excellent tolerance and future research at our institution will focus on the long term outcome following repeated treatment with high activities. If phase II clinical trials confirm the encouraging results that were reported in the feasibility studies, ^{188}Re-HDD/Lipiodol should be compared to the standard palliative treatment for HCC (TACE) in a randomized controlled trial. Subgroups of patients that did not benefit from the radionuclide therapy should be identified (role of underlying liver dysfunctions, general status, tumor dimensions, …).
Research concerning various dosimetry and radiation protection matters is ongoing at our hospital, including patient-specific calculation of the tumor doses based on SPECT-scans and estimation of the radiation burden for staff and relatives of patients treated with ^{188}Re-HDD/Lipiodol.
In patients in whom local ablative techniques are applicable, combining RFA or PEI with locoregional radionuclide therapy might be of added value. Combining radionuclide therapy with systemic or locoregional chemotherapy could also enhance the antitumoral effects. In the present work, a number of cytostatic drugs that might have a role to play in management of HCC were tested *in vitro* for synergistic effects when combined with exposure to ^{188}Re. Results so far using topotecan, 5-fluorouracil and gemcitabin were not encouraging since synergistic effects were lacking or markedly less pronounced than observed with external beam irradiation. Future experiments should elucidate whether other drugs such as taxanes and anthracyclines yield more promising results when combined with ^{188}Re.

In conclusion, a considerable number of clinical trials, directed to the application of ^{188}Re in oncological as well as benign conditions, have demonstrated its feasibility. However, most clinical know-how remains limited to a number of expert centers in Germany and Asia. In the future, efforts should be made to facilitate innovative research using a ^{188}W/^{188}Re-generator and the implementation of ^{188}Re based therapies should be encouraged in other centers.

References

1. Llovet JM, Bruix J. Systematic review of randomized trials for unresectable hepatocellular carcinoma: Chemoembolization improves survival. *Hepatology* 2003;37:429-442.
2. Camma C, Schepis F, Orlando A, et al. Transarterial chemoembolisation for unresectable hepatocellular carcinoma: Meta-analysis of randomised controlled trials. *Radiology* 2002;224:47-54.
3. Reidy DL, Schwartz JD. Therapy for unresectable hepatocellular carcinoma: review of the randomized clinical trials-I: hepatic arterial embolization and embolization-based therapies in unresectable hepatocellular carcinoma. *Anticancer Drugs* 2004;15:427-37.
4. Raoul JL, Guyader D, Bretagne JF, et al. Prospective randomized trial of chemoembolization versus intraarterial injection of 131I-labeled iodized oil in the treatment of hepatocellular carcinoma. *Hepatology* 1997;26:1156-1161.
5. Ho S, Lau WY, Leung TWT and Johnson PJ. Internal radiation therapy for patients with primary or metastatic hepatic cancer. *Cancer* 1998; 83: 1894-1907.
6. Risse JH, Grünwald F, Kersjes W, et al. Intraarterial HCC Therapy with I-131-Lipiodol. *Cancer Biother Radiopharm* 2000;15:65-70.
7. Rindani R, Hugh T, Roche J, Roach PJ, Smith RC. 131I Lipiodol therapy for unresectable hepatocellular carcinoma. *ANZ J Surg* 2002;72:210-214.
8. Bhattacharya S, Novell JR, Dusheiko GM, Hilson AJ, Dick R, Hobbs KE. Epirubicin-Lipiodol chemotherapy versus ^{131}Iodine-Lipiodol radiotherapy in the treatment of unresectable hepatocellular carcinoma. *Cancer* 1995;76:2202-2210.
9. Raoul JL, Guyader D, Bretagne JF, et al. Randomized controlled trial for hepatocellular carcinoma with portal vein thrombosis: intra-arterial iodine-131-iodized oil versus medical support. *J Nucl Med* 1994;35:1782-1787.
10. de Baere T, Taourel P, Tubiana JM, Kuoch V, Ducreux M, Lumbroso J, Roche AJ. Hepatic intraarterial 131I iodized oil for treatment of hepatocellular carcinoma in patients with impeded portal venous flow. *Radiology* 1999;212:665-668.
11. Monsieurs M, Bacher K, Brans B, et al. Patient dosimetry for 131-I-Lipiodol therapy*. Eur J Nucl Med Mol Imaging* 2003;30:554-561.
12. Pugh RN, Murray-Lyon IM, Dawson JL, Pietroni MC, Williams R. Transection of the oesophagus for bleeding oesophageal varices. *Br J Surg* 1973;60:646-649.
13. Lee YS, Jeong JM, Kim YJ, Chung JW, Park JH, Suh YG, et al. Synthesis of 188 Re-labelled long chain alkyl diaminedithiol for therapy of liver cancer. *Nucl Med Commun* 2002;23:237-242.
14. Sundram FX, Jeong J-M, Zanzonico P, Bernal P, Chau T, Onkhuudai P, et al. Trans-arterial Rhenium-188 Lipiodol in the treatment of inoperable hepatocellular carcinoma. An IAEA sponsored multi-centre phase 1 study. *World J Nucl Med* 2002;1:5-11.

15. Lambert B, Bacher K, Defreyne L, Gemmel F, Van Vlierberghe H, Jeong JM, et al.. 188Re-HDD/Lipiodol therapy for hepatocellular carcinoma: a phase I clinical trial. *J Nucl Med* 2005;46:60-66.
16. Raoul JL, Bourguet P, Bretagne JF, et al. Hepatic artery injection of I-131-labeled Lipiodol. Biodistribution study results in patients with hepatocellular carcinoma and liver metastases. *Radiology* 1988;168:541-545.
17. Nakajo M, Kobayashi H, Shimabukuro K, et al. Biodistribution and in vivo kinetics of iodine- 131 Lipiodol infused via the hepatic artery of patients with hepatic cancer. *J Nucl Med* 1988;29:1066-1077.
18. Madsen MT, Park CH, Thakur ML. Dosimetry of iodine-131 ethiodol in the treatment of hepatoma. *J Nucl Med* 1988;29:1038-1044.
19. Sundram F, Chau TC, Onkhuudai P, Bernal P, Padhy AK. Preliminary results of transarterial rhenium-188 HDD Lipiodol in the treatment of inoperable primary hepatocellular carcinoma. *Eur J Nucl Med Mol Imaging* 2004;31:250-257.
20. Bruix J, Sherman M, Llovet JM, et al. Clinical management of hepatocellular carcinoma. Conclusions of the Barcelona-2000 EASL conference. European Association for the Study of the Liver. *J Hepatol* 2001;35:421-430.
21. Boschi A, Uccelli L, Duatti A, Colamussi P, Cittanti C, Filice A, et al.. A kit formulation for the preparation of 188Re-Lipiodol: preclinical studies and preliminary therapeutic evaluation in patients with unresectable hepatocellular carcinoma. *Nucl Med Commun* 2004;25:691-699.
22. Garin E, Noiret N, Malbert C, Lepareur N, Roucoux A, Caulet-Maugendre S, et al.. Development and biodistribution of ^{188}Re-SSS Lipiodol following injection into the hepatic artery of healthy pigs. *Eur J of Nucl Med Mol Imaging* 2004;3:542-546.
23. Houle S, Yip TK, Shepherd FA, et al. Hepatocellular carcinoma: pilot trial of treatment with Y-90 microspheres. *Radiology* 1989;172:857-860.
24. Shepherd FA, Rotstein LE, Houle S, Yip TC, Paul K, Sniderman KW. A phase I dose escalation trial of yttrium-90 microspheres in the treatment of primary hepatocellular carcinoma. *Cancer* 1992;70:2250-2254.
25. Carr BI. Hepatic arterial ^{90}Ytrrium glass microspheres (Therasphere) for unresectable hepatocellular carcinoma: Interim safety and survival data on 65 patients. *Liver Transpl* 2004;10:S107-S110.
26. Geschwind JF, Salem R, Carr BI, et al. Yttrium-90 microspheres for the treatment of hepatocellular carcinoma. *Gastroenterology* 2004;127:S194-205.
27. Lau WY, Leung WT, Ho S, et al. Treatment of inoperable hepatocellular carcinoma with intrahepatic arterial yttrium-90 microspheres: a phase I and II study. *Br J Cancer* 1994;70:994-999.
28. Goin JE, Salem R, Carr BI, et al. Treatment of unresectable hepatocellular carcinoma with intrahepatic yttrium 90 microspheres: a risk-stratification analysis. *J Vasc Interv Radiol* 2005;16:195-203.
29. Goin JE, Salem R, Carr BI, et al. Treatment of unresectable hepatocellular carcinoma with intrahepatic yttrium 90 microspheres: factors associated with liver toxicities. *J Vasc Interv Radiol* 2005;16:205-213.

30. Ho S, Lau WY, Leung TW, Chan M, Johnson PJ, Li AK. Clinical evaluation of the partition model for estimating radiation doses from yttrium-90 microspheres in the treatment of hepatic cancer. *Eur J Nucl Med* 1997;24:293-298.
31. Leung TW, Lau WY, Ho SK, et al. Radiation pneumonitis after selective internal radiation treatment with intraarterial 90 yttrium-microspheres for inoperable hepatic tumours. *Int J Radiat Oncol Biol Phys* 1995;33:919-924.
32. Salem R, Lewandowski R, Roberts C, et al. Use of Yttrium-90 Glass Microspheres (TheraSphere) for the Treatment of Unresectable Hepatocellular Carcinoma in Patients with Portal Vein Thrombosis. *J Vasc Interv Radiol* 2004;15:335-345.
33. Liu L, Jiang Z, Teng GJ, et al. Clinical and experimental study on regional administration of phosphorus 32 microspheres in treating hepatic carcinoma. *World J Gastroenterol* 1999;5:492-505.
34. Wunderlich G, Drews A, Kotzerke J. A kit for labeling of 188Re human serum albumin microspheres for therapeutic use in nuclear medicine. *Appl Radiat Isot* 2005;62:915-918.
35. Kotzerke J, Liepe K, Brogsitter C, Wunderlich G, Leonhard J, Folprechts G, Pinkert J. First results of Re188-microspheres in selective hepatic radioembolisation. *J Nucl Med* 2005;46:449P.
36. Häfeli UO, Casillas S, Dietz DW, Pauer GJ, Rybicki LA, Conzone MS, Day DE. Hepatic tumour radioembolization in a rat model using radioactive rhenium (Re-186/Re-188) glass microspheres. *Int J Radiat Oncol Biol Phys* 1999;44:189-1999.
37. Häfeli UO, Roberts WK, Pauer GJ, Kraeft ST, Macklis RM. Stability of biodegradable radioactive rhenium (Re-186 and Re-188) microspheres after neutron-activation. *Appl Radiat Isot* 2001;54:869-879.
38. Nijsen F, Rook D, Brandt C, et al. Targeting of liver tumour in rats by selective delivery of holmium-166 loaded microspheres: a biodistribution study. *Eur J Nucl Med* 2001;28:743-749.
39. Schwartz JD, Schwartz M, Mandeli J, Sung M. Neoadjuvant and adjuvant therapy for resectable hepatocellular carcinoma: review of the randomised clinical trials. *Lancet Oncol* 2002;3:593-603.
40. Lau WY, Leung TW, Ho SK, et al. Adjuvant intra-arterial iodine-131-labelled Lipiodol for resectable hepatocellular carcinoma: a prospective randomised trial. *Lancet* 1999;353:797-801.
41. Muto Y, Moriwaki H, Ninomiya M, et al. Prevention of second primary tumors by an acyclic retinoid, polyprenoic acid, in patients with hepatocellular carcinoma. *N Eng J Med* 1996;334:1561-1567.
42. Abrams RA, Pajak TE, Pajak TF, et al. Survival results among patients with alpha-fetoprotein-positive, unresectable hepatocellular carcinoma: analysis of three sequential treatments of the RTOG and Johns Hopkins oncology center. *Cancer Journal Am Sci* 1998;4:178-184.
43. Brans B, Van Laere K, Gemmel F, et al. Combining iodine-131 Lipiodol therapy with low-dose cisplatin as a radiosensitiser: preliminary results in hepatocellular carcinoma. *Eur J Nucl Med Mol Imaging* 2002;29:928-932.

44. Maeda H. SMANCS and polymer-conjugated macromolecular drugs: advantages in cancer chemotherapy. *Adv Drug Deliv Rev* 2001;46:169-185.
45. Yi SW, Kim YH, Kwon IC, et al. Stable Lipiodolized emulsions for hepatoma targeting and treatment by transcatheter arterial chemoembolisation. *J Control Release* 1998;50:135-143.
46. Yoon CJ, Chung JW, Park JH, et al. Transcatheter arterial chemoembolisation with paclitaxel-Lipiodol solution in rabbit VX liver Tumor. *Radiology* 2003;229:126-131.
47. Al-Mufti RA, Pedley RB, Marshall D, Begent RH, Hilson A, Winslet MC, Hobbs KE. In vitro assesment of Lipiodol-targeted radiotherapy for liver and colorectal cancer cell lines. *Br J Cancer* 1999;79:1665-1671.
48. Knapp FF. Rhenium -188- A generator- derived radioisotope for cancer therapy. *Cancer Biother Radiopharm* 1998;13:337-349.
49. Palmedo H, Guhlke S, Bender H, Sartor J, Schoeneich G, Risse J et al.. Dose escalation study with rhenium-188 hydroxyethilidene diphosphonate in prostate cancer patients with osseous metastases. *Eur J Nucl Med* 2000; 27: 123-130.
50. Maxon HR, Schroder LE, Washburn LC, Thomas SR, Samaratunga RC, Biniakiewicz D, et al. Rhenium-188(Sn)HEDP for treatment of osseous metastases. *J Nucl Med* 1998;39:659-663.
51. Lin WY, Lin CP, Yeh SJ, Hsieh BT, Tsai ZT, Ting G, et al. Rhenium-188 hydroxyethylidene diphosphonate: a new generator-produced radiotherapeutic drug of potential value for the treatment of bone metastases. *Eur J Nucl Med* 1997;24:590-595.
52. Liepe K, Runge R and Kotzerke J. The benefit of bone-seeking radiopharmaceuticals in the treatment of metastatic bone pain*. J Cancer Res Clin Oncol* 2005;131:60-66.
53. Palmedo H, Manka-Waluch A, Albers P, Schmidt-Wolf IG, Reinhardt M, Ezziddin S, et al. Repeated bone-targeted therapy for hormone refractory prostate carcinoma: randomized phase II clinical trial with the new high energetic radiopharmaceutical Rhenium-188 Hydroxyethylidenediphosphonate. *J Clin Oncol* 2003; 21: 2869-2875.
54. O'Sullivan JM, McCready VR, Flux G, Norman AR, Buffa FM, Chittenden S, et al.. High activity Rhenium-I86 HEDP with autologous peripheral blood stem cell rescue: a phase I study in progressive hormone refractory prostate cancer metastatic to bone. *Br J Cancer* 2002; 86:1715-1720.
55. Zamora PO, Guhlke S, Bender H, Diekman D, Rhodes BA, Biersack H-J, Knapp FFJr. Experimental Radiotherapy of receptor- positive Prostate Adenocarcinoma with 188-Re-RC-160, a directly-radiolabeled somatostatin analogue. *Int J Cancer* 1996;65:214-220.
56. Zamora PO, Bender H, Knapp FF, Rhodes BA, Biersack HJ. Targeting peptides for pleural cavity tumor radiotherapy: specificity and dosimetry of Re-188-RC-160. *Hybridoma* 1997;16:85-91.
57. Zamora PO, Bender H, Gulhke S, Marek MJ, Knapp FF Jr, Rhodes BA, Biersack HJ. Pre-clinical experience with Re-188-RC-160, a radiolabeled somatostatin analog for use in peptide-targeted radiotherapy. *Anticancer Res* 1997;17:1803-1808.

58. Safavy A, Khazaeli MB, Qin H, Buchsbaum DJ. Synthesis of bombesin analogues for radiolabeling with rhenium-188. *Cancer* 1997;80:2354-2359.
59. Luo TY, Hsieh BT, Wang SJ, Lin WY, Lee TW, Shen LH, Su MJ. Preparation and biodistribution of Rhenium-188ECD/Lipiodol in rats following hepatic arterial injection. *Nucl Med Biol* 2004;31:671-677.

Summary
Samenvatting
Résumé

Summary

Hepatocellular Carcinoma or HCC is the most prevalent primary liver cancer. It constitutes the third cause of cancer related deaths, responsible for more than 500.000 deaths worldwide annually. Due to the underlying carcinogenic risk factors such as chronic viral hepatitis and alcohol intake, the vast majority of patients presenting with HCC, suffer from cirrhosis, a condition limiting the therapeutic possibilities. Surgery, either by means of hepatectomy or liver transplantation, is the mainstay of curative treatment. Overall, if the degree of liver dysfunction and the tumor load is taken into consideration, the vast majority of patients is not eligible for surgery.
Amongst the non-surgical treatment modalities producing response rates exceeding 20% are the use of Lipiodol mixed with chemotherapeutic agents or radiolabeled with Iodine-131 (^{131}I) without subsequent embolization. Encouraging results have been obtained using ^{131}I-Lipiodol, but the use of ^{131}I has hampered its routine clinical implementation. ^{131}I has a physical half-life of 8 days and emits a high energetic gamma(γ)-ray (364 keV, with an abundancy of 82%) necessitating prolonged hospitalization for radioprotection purposes. Although ^{131}I-Lipiodol therapy is generally well tolerated, a 'dose escalation' study was not conducted so far as the administration of activities exceeding 2.22 GBq is restricted by the above mentioned practical issues. Rhenium-188 (^{188}Re) has favorable characteristics for radionuclide therapy and considering the limited success of ^{131}I-Lipiodol for treatment of relatively large tumors, the switch towards a radionuclide with a higher energy of the beta(β)-emission (2120 keV and 1960 keV for ^{188}Re versus 606 keV for ^{131}I) might yield improved response rates. ^{188}Re emits a γ-ray of 155 keV at an abundancy of 15%, allowing γ-camera imaging and it has a relatively short physical half-life of 17 hours, limiting radiation protection problems. Additionally, the radionuclide is eluted from a ^{188}W/^{188}Re-generator, which has a long useful shelf life of several months and provides a good yield of carrier-free ^{188}Re routinely.

In the present work we aimed at conducting a feasibility study in which the biodistribution and safety was investigated following the administration of 3.7 GBq ^{188}Re-4-hexadecyl-1-2,9,9-tetramethyl-4,7-diaza-1,10-decanethiol/Lipiodol (^{188}Re-HDD/Lipiodol) in the hepatic artery. A fast blood clearance of the injected activity was observed with a calculated effective half-life of 7.6 (± 2.2 SD) hours in blood. The predominant elimination of the activity was through urinary excretion with a mean renal clearance of 44.1 (± 11.7 SD) % of the injected activity within the 76 hours following administration. The absorbed dose to the liver (including the tumor), the lungs, the kidneys and the thyroid was 4.5 ± 1.9 Gy, 4.1 ± 1.2 Gy, 0.9 ± 0.7 Gy and 0.3 ± 0.1 Gy, respectively. The calculated whole body effective half-life was 14.3 (± 0.9 SD) hours. Treatment was well tolerated, in particular in patients with well compensated cirrhosis and according to the dosimetric estimations a further activity escalation seemed feasible.

However, in patients suffering moderately advanced cirrhosis, the administration of 3.7 GBq ^{188}Re-HDD/Lipiodol did not yield encouraging results and therefore, it was preferred to conduct the activity escalation study in the subset of patients with well compensated cirrhosis. Activities up to 7.0 GBq ^{188}Re-HDD/Lipiodol were administered without the occurrence of unacceptable adverse events. The most important drawback consisted of the limited efficiency of the radiolabeling procedure, which implicates careful planning to implement such high activities in routine practice. In addition, attention should be paid in the future to patients developing pulmonary symptoms, such as a cough or dyspnea. In our overall experience, 2 out of 39 assessable patients developed unexplained prolonged dyspnea requiring corticosteroid treatment.

In the second part of our clinical research, a retrospective analysis of our experiences with radiolabeled Lipiodol for patients awaiting liver transplantation for HCC was performed. Two patients died while on the waiting list (10%) and 4 out of 20 transplanted patients (20%) suffered recurrent disease. In 5 out of 22 explants necrosis exceeded 90% and these results compare favorably with a historical control group from our hospital. The overall recurrence free survival was 19.7 months (range 1.75-56) with a mean follow-up of 20.1 months.
These data support the evaluation on larger patient numbers to confirm the benefit of radiolabeled Lipiodol in candidates for liver transplantation suffering HCC.

In the preclinical study, we aimed at developing a three dimensional *in vitro* model for HCC, which would be a helpful tool in our search for strategies to optimize radionuclide therapy for HCC. Multicellular tumor aggregates or "spheroids" of HepG2-cells were cultured and exposed to high dose rate γ-irradiation in combination with cisplatin, gemcitabine or 5-fluorouracil. Toxicity was evaluated by means of comparative growth curves, an outgrowth assay and histology. Supra-additive effects lasting for 4 weeks were observed for all drugs tested in combination with 10 Gy. In a subsequent experiment, the effects of low dose rate irradiation (^{188}Re) were compared with high dose rate γ-irradiation, with or without chemotherapy (gemcitabine, 5-fluorouracil or topotecan) added. For ^{188}Re, supra-additive effects were less pronounced and only evident with 5-fluorouracil and gemcitabine but not with concomitant exposure to topotecan. Only in case of gemcitabine the supra-additive effects with low dose rate irradiation lasted till week 4.

Samenvatting

Het hepatocellulair carcinoom of HCC is de meest prevalente primaire kwaadaardige levertumor. Het is wereldwijd de derde meest frequente oorzaak van sterfte tengevolge van kanker, verantwoordelijk voor meer dan een half miljoen overlijdens per jaar. Chronische virale hepatitis en alcohol gebruik zijn belangrijke carcinogene risicofactoren. De meeste patiënten bij wie de diagnose van een HCC gesteld wordt, hebben dan ook een cirrotische lever. Dit onderliggend leverlijden betekent voor veel patiënten een ernstige beperking van de therapeutische mogelijkheden. Heelkunde, onder de vorm van een partiële leverresectie of een transplantatie, is de beste optie met het oog op definitieve genezing. Helaas is een heelkundige behandeling in de meerderheid van de patiënten niet aangewezen omdat de onderliggende cirrose te ernstig is of omdat de tumor te uitgebreid is.
Er zijn verscheidene niet-heelkundige behandelingsmodaliteiten toepasbaar bij patiënten die niet in aanmerking komen voor chirurgie. Slechts een beperkt aantal van deze strategieën leidt echter tot een aanvaardbaar aantal (20%) goede responsen. Tot deze groep behoort de lokoregionale behandeling met Lipiodol gemengd met chemotherapeutica of radioactief gemerkt met Jodium-131 (^{131}I). Er werden bemoedigende resultaten bekomen met ^{131}I-Lipiodol, maar helaas wordt deze behandeling niet wijd toegepast tengevolge van de radioprotectieve beperkingen verbonden aan het gebruik van ^{131}I. ^{131}I heeft een fysisch half leven van 8 dagen en zendt een hoog energetische gamma(γ)-straal uit (364 keV, met een abundantie van 82%) waardoor een isolatie in een aangepaste radionuclidenkamer gedurende minstens vijf dagen noodzakelijk is. Ondanks de goede tolerantie van een behandeling met ^{131}I-Lipiodol kon toch geen 'dosis escalatie' studie uitgevoerd worden: de toediening van activiteiten boven de 2.22 GBq zou gepaard gaan met een onaanvaardbaar lange opname in isolatie. Rhenium-188 (^{188}Re) heeft geschikte fysische karakteristieken voor radionuclidentherapie. De hogere energie van de beta(β)-emissies (2120 keV en 1960 keV voor ^{188}Re versus 606 keV voor ^{131}I) kan een voordeel betekenen in de behandeling van relatief grote tumoren. In deze subgroep van tumoren was het effect van behandeling met ^{131}I-Lipiodol immers weinig succesvol. ^{188}Re zendt eveneens een γ-straal van 155 keV uit met een abundantie van 15%, hetgeen beeldvorming aan de hand van scintigrafie mogelijk maakt. De lagere energie en abundantie van de γ-straling, alsook het relatief korte fysisch half leven van 17 uur, maken van ^{188}Re een aantrekkelijk radionuclide op radioprotectief vlak. Bovendien wordt dit radionuclide bekomen via elutie van een ^{188}W/^{188}Re-generator. Deze generator kan gebruikt worden in het ziekenhuis en kan gedurende verscheidene maanden 'carrier-vrij' ^{188}Re voorzien met het oog op therapeutische toepassingen.

In het kader van dit proefschrift werd een fase I studie opgezet om de biodistributie en veiligheid van het gebruik van 3.7 GBq ^{188}Re-4-hexadecyl-1-2,9,9-tetramethyl-4,7-diaza-1,10-decanethiol/Lipiodol (^{188}Re-HDD/Lipiodol) in kaart te brengen. Het radiofarmacon werd toegediend in de arteria hepatica propria. Een snelle klaring van de activiteit in het bloed werd waargenomen met een effectief half leven van 7.6 (± 2.2 SD) uur. De activiteit werd hoofdzakelijk

uitgescheiden via de urine met een gemiddelde renale klaring van 44.1 (± 11.7 SD) %, 76 uur na toediening. De geabsorbeerde dosis ter hoogte van de lever (inclusief de tumor), de longen, de nieren en de schildklier bedroeg 4.5 ± 1.9 Gy, 4.1 ± 1.2 Gy, 0.9 ± 0.7 Gy en 0.3 ± 0.1 Gy, respectievelijk. Het effectief half leven voor het totale lichaam werd geschat op 14.3 (± 0.9 SD) uur. De behandeling werd uitstekend verdragen, in het bijzonder bij patiënten die een goed gecompenseerde leverdysfunctie (Child-Pugh A) hadden. In deze subgroep patiënten leek een verder opdrijven van de toegediende activiteit haalbaar.
In een daaropvolgende studie werden activiteiten van 4.9 tot 7.0 GBq ^{188}Re-HDD/Lipiodol trapsgewijs toegepast bij patiënten met een goed gecompenseerde leverdysfunctie. Er werden geen onaanvaardbare bijwerkingen gedocumenteerd. Verder opdrijven van de toegediende activiteit werd beperkt door de relatief lage labelingsefficiëntie van de synthese van het radiofarmacon. Bij patiënten met ernstiger onderliggend leverlijden (Child-Pugh B), waren de resultaten na toediening van 3.7 GBq ^{188}Re-HDD/Lipiodol echter niet bemoedigend en er werd dan ook afgezien van een verder opdrijven van de toegediende activiteiten in deze patiënten.
Verder wensen wij erop te wijzen dat er in de toekomst aandacht dient besteed te worden aan patiënten die hoest of kortademigheid ontwikkelen na behandeling met ^{188}Re-HDD/Lipiodol. In onze ervaring traden er longklachten op bij 2 op 39 patiënten en was behandeling met corticosteroïden aangewezen in deze gevallen.

In het tweede deel van het klinisch onderzoek verricht in het kader van dit proefschrift, werd een retrospectieve analyse uitgevoerd van de patiënten die op onze afdeling behandeld werden met radioactief Lipiodol terwijl zij op de wachtlijst geregistreerd waren voor een lever transplantatie. Twee patiënten overleden op de wachtlijst (10%) en 4 van de 20 getransplanteerde patiënten (20%) heeft nadien een recidief in de donorlever of metastasen ontwikkeld. In 5 van de 22 explanten overschreed het percentage necrose 90% van de tumor. Een dergelijke uitgebreide necrose werd niet waargenomen in een historische controle groep, bestaande uit niet-voorbehandelde patiënten die een levertransplantatie omwille van HCC ondergingen in ons ziekenhuis. In de groep patiënten behandeld met radioactief Lipiodol bedroeg de ziektevrije overleving gemiddeld 19.7 maanden (range 1.75-56, met een gemiddelde observatieduur van 20.1 maanden). Deze positieve bevindingen dienen echter nog verder bevestigd te worden door een groter aantal transplantkandidaten met een HCC te behandelen met radioactief gemerkt Lipiodol.

In de preklinische studie, werd een drie dimensionaal *in vitro* model voor HCC, bestaande uit multicellulaire tumor aggregaten of "sferoïden" van HepG2-cellen, ontwikkeld. Een dergelijk model maakt het mogelijk om op eenvoudige wijze de efficiëntie van een aantal strategieën ter optimalisatie van radionuclidentherapie voor HCC te testen. De HepG2-sferoïden werden blootgesteld aan een γ-bestraling met hoog dosistempo gecombineerd met cisplatinum, gemcitabine of 5-fluorouracil. De toxiciteit werd geëvalueerd aan de hand van vergelijkende uitgroeicurves en histologie. Supra-additieve effecten die langer dan 4 weken

aanhielden, werden geobserveerd voor alle geteste chemotherapeutica. In een daaropvolgend experiment, werden de effecten van bestraling met behulp van ^{188}Re (laag dosistempo) vergeleken met deze van de γ-bestraling aan hoog dosistempo, al dan niet gecombineerd met cytostatica (gemcitabine, 5-fluorouracil of topotecan). Voor ^{188}Re waren de supra-additieve effecten minder uitgesproken en enkel aantoonbaar voor 5-fluorouracil en gemcitabine. Concomitant gebruik van ^{188}Re en topotecan induceerde geen supra-additief effect. Enkel in het geval van gemcitabine waren de supra-additieve effecten na bestraling met laag dosistempo nog aantoonbaar na vier weken.

Résumé

Le carcinome hépatocellulaire (CHC) est la forme de cancer hépatique ayant la plus forte prévalence. Mondialement responsable de plus de 500.000 décès annuels, il occupe la troisième place parmi les décès par maladies liées au cancer. En raison des risques carcinogènes sous-jacents, tels que l'hépatite virale chronique et la consommation d'alcool, la grande majorité des patients présentant un carcinome CHC souffre de cirrhose, ce qui limite les possibilités thérapeutiques. La chirurgie, hepatectomie ou transplantation, reste l'option préférée, assurant les meilleures conditions de guérison. Mais si le degré de dysfonctionnement du foie et la tumeur sont pris en compte, la grande majorité des patients ne subira pas d'acte chirurgical.

Seul un nombre restreint de stratégies offre un taux satisfaisant de réussite, excédant 20%. Parmi celles-ci le Lipiodol, combiné à des substances chimiothérapeutiques ou radiomarqué avec de l'Iode-131 (^{131}I). Des résultats encourageants furent obtenus avec le ^{131}I-Lipiodol, mais la radioprotection nécessaire en restreint l'utilisation. En effet, ^{131}I émet un fort rayonnement γ (364 keV; abondance 82 %) nécessitant la mise en quarantaine du patient en chambre radionuclide durant un minimum de 5 jours par mesure de sécurité. Malgré une bonne tolérance au traitement avec le ^{131}I-Lipiodol, une étude 'à dose progressive' n'a pu être effectuée en raison de la quarantaine, inacceptable quant à la durée, pour une irradiation supérieure à 2.22 GBq. Le Rhenium-188 (^{188}Re) présente des caractéristiques physiques adéquates pour une thérapie radionucléaire. Les énergies de rayonnement β plus élevées (2120 keV et 1960 keV pour ^{188}Re comparé à 606 keV pour ^{131}I) sont notoirement plus efficaces dans le traitement de tumeurs relativement importantes. L'émission γ du Rhenium-188 (155 keV; abondance de 15 %) permet d'obtenir des images par scintigraphie. L'énergie moindre et l'abondance de rayons gammas, ainsi que la courte période (17 heures) font du ^{188}Re un radionuclide intéressant au point de vue radioprotection. De plus, le radionuclide peut être élué de façon routinière avec un bon rendement à partir d'un générateur ^{188}W/^{188}Re.

Cette étude présente d'abord une étude de faisabilité incluant la biodistribution de et la sureté du traitement après injection de 3.7 GBq ^{188}Re-4-hexadecyl-1-2,9,9-tetramethyl-4,7-diaza-1,10-decanethiol/Lipiodol (^{188}Re-HDD/Lipiodol) dans *l'Arteria hepatica propria*. Une rapide clairance de l'activité sanguine fut observée dont la période calculée est de 7.6 (± 2.2 DS) heures. L'activité fut éliminée principalement par les reins. La clairance rénale est de 44.1 (±11,7 DS)% , 76 heures après administration. La dose absorbée par le foie (tumeur incluse), par les poumons, les reins et la thyroïde est respectivement de 4.5 ± 1.9 Gy, 4.1 ± 1.2 Gy, 0.9 ± 0.7 Gy et 0.3 ± 0.1 Gy. La période totale, calculée d'après les données mesurées, est de 14.3 (±0.9 DS) heures. Le traitement fut très bien supporté, en particulier par les patients souffrant d'un dysfonctionnement hépatique bien compensé (Child-Pugh A). Ce sous-groupe de patients semblerait bien tolérer une activité supérieure.

L'administration de 3.7 GBq ^{188}Re-HDD/Lipiodol aux patients atteints d'une cirrhose modérée n'a par contre pas fourni de résultats encourageants en conséquence de quoi nous avons préféré diriger notre recherche vers les patients atteints de cirrhose bien compensée.
Des activités atteignant 7.0 GBq ^{188}Re-HDD/Lipiodol furent administrées sans effets contraires notoires. Des expériences avec des activités supérieures furent limitées par la relative inefficacité du radiomarquage dans la synthèse de la substance radiothérapeutique. Par ailleurs, notre expérience nous a fait attacher une importance particulière aux patients développant des symptômes pulmonaires tels que la toux ou une dyspnée suite au traitement. Deux des trente-neuf patients ont développé une inexplicable dyspnée récalcitrante nécessitant un traitement corticoïde.

Le second volet de notre recherche clinique traite de l'analyse des résultats de nos expériences avec le Lipiodol radiomarqué sur des patients souffrant de CHC et attendant une transplantation de foie. Deux patients (10%) sont décédés en attente d'une transplantation; 4 des 20 transplantés (20%) ont souffert d'une malignité récurrente. Dans cinq des vingt-deux foies la nécrose excédait 90% de la tumeur. Une nécrose aussi importante n'est pas retrouvée chez le groupe de contrôle historique, composé de patients de notre hôpital. La durée moyenne d'absence de rechute est de 19.7 mois (min. 1.75–max. 56); les suivi moyen est de 20.1 mois par patient.
Ces données appellent une étude à plus grande échelle afin de confirmer l'effet bénéfique du Lipiodol radiomarqué chez les patients CHC attendant une transplantation.

Lors de l'étude pré-clinique nous avons développé un modèle *in-vitro* tridimensionnel, pouvant offrir un outil à l'optimalisation de la thérapie radionuclide. Des 'sphéroïdes' ou agrégats multicellulaires tumoraux de cellules HepG2 furent mis en culture et exposés à une importante radiation γ combiné soit à du cisplatin, de la gemcitabine ou a du 5-fluorouracil. La toxicité a été évaluée en utilisant et les courbes comparatives de croissance en 3D, la mise en culture en 2D et l'histologie. Pour toutes les chimiothérapies combinées à une irradiation de 10 Gy (Cobalt 60), des effets supra-additifs furent observés durant plus de 4 semaines. Ensuite, nous avons comparé l'effet d'une irradiation à faible débit de dose avec le ^{188}Re à l'effet d'une irradiation à un haut débit de dose (γ, Cobalt 60) en combinaison avec ou sans chimiothérapie (gemcitabine, 5-fluorouracil ou topotecan). En ce qui concerne le ^{188}Re, les effets supra-additifs furent moins prononcés et uniquement détectable avec le 5-fluorouracil et la gemcitabine. Utilisation concomitante de ^{188}Re et topotecan n'a induit nul effet supra-additif. Avec la gemcitabine, les effets supra-additifs après radiation étaient manifestes après 4 semaines.

Addenda

Curriculum vitae

Personal

Name: Lambert
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Education § Training

1987-1993: Regina Caeli Lyceum, Rozenlaan 45, 1700 Dilbeek
1993-2001: Faculty of Medicine, Ghent University, De Pintelaan 185 K3, Belgium
November 1998-May 1999: Clinical training in Sucre, Bolivia (Instituto Politecnico TK, Hospital de la Mujer, Clinica Universitaria de Santa Barbara)
June 2001: Medical doctor, Ghent University, with additional certification in electrocardiography.
October 2001-September 2003: Postgraduate course in Nuclear Medicine
October 2001-September 2003: Postgraduate course in Internal Medicine
April 2004: Qualification in Good Clinical Practice

Experience

Since October 2001: PhD student at the Nuclear Medicine Division, University Hospital Ghent, Belgium, with a project entitled: "Rhenium-188-Lipiodol: second generation radionuclide therapy for Hepatocellular Carcinoma". Grant: research mandate of the Ghent University Hospital: BOF (011D 9501).

Membership

Member of the Belgian Association of Nuclear Medicine, Radionuclide Therapy Task Group
Member of the E-SIOP Nuclear Medicine Subcommittee
Member of the European Association of Nuclear Medicine

Awards

Best Poster Award: Belgisch Genootschap Nucleaire Geneeskunde, Knokke May 25th, 2003. Comparison of the cytotoxic effect of 131I-Lipiodol therapy and 188Re-Lipiodol therapy in HCC patients. K. De Ruyck, A. Vral, B. Lambert, R.A. Dierckx, H. Thierens.

Best Clinical Poster Award: Belgisch Genootschap Nucleaire Geneeskunde, Knokke May 21st, 2005. 188Re-HDD/Lipiodol Therapy for Hepatocellular Carcinoma: An Activity Escalation Study. B. Lambert, F. De Vos, E. Vrancken, K. Bacher, L. Defreyne, H. Van Vlierberghe, J.M. Jeong, J. Van Meerbeeck, P. Smeets, H. Thierens, C. Van de Wiele.

Languages

Dutch, French, English and basic knowledge of Spanish

List of publications

Publications in peer reviewed international journals

Neo-adjuvant treatment of hepatocellular carcinoma: antitumoral activity of intra-arterial 131-I-Lipiodol therapy. B. Brans, F. De Winter, L. Defreyne, R. Troisi, B. Lambert, P. Vanlangenhove, H. Van Vlierberghe, M. Praet, B. de Hemptinne, R.A. Dierckx. Cancer Biother Radiopharm 2001;16:333-338.

High 18F-fluorodeoxyglucose uptake in paraspinal textiloma. F. De Winter, W. Huysse, P. De Paepe, B. Lambert, B. Poffyn, R.A. Dierckx. Clin Nucl Med 2002;27:132-133.

Quality of Life assessment in radionuclide therapy: a feasibility study of eortcQLQ-C30 questionnaire in palliative I-131-Lipiodol therapy. B. Brans, B. Lambert, F. De Winter, S Van Belle, H Van Vlierberghe, B. de Hemptinne, R.A Dierckx. Eur J Nucl Med Mol Imaging 2002;29:1374-1379.

18FDG PET versus high dose 67Ga scintigraphy for restaging and treatment follow-up of lymphoma patients. B. Van Den Bossche, B. Lambert, F. De Winter, A. Kolindou, R.A. Dierckx, L. Noens, C. Van de Wiele. Nuclear Med Com 2002;23:1079-1083.

Comparison of the cytotoxic effect of 131-I-Lipiodol therapy and 188-Re-Lipiodol therapy in HCC patients. K. De Ruyck, B. Lambert, K. Bacher, F. Gemmel, F. De Vos, A. Vral, R.A. Dierckx and H. Thierens. J Nucl Med 2004;45:612-618.

Radionuclide Detection of Peritoneovenous Shunt Patency. F. Gemmel, M. Ravier, R. Troisi, H. Van Vlierberghe, B. Lambert, V. Renard, R.A. Dierckx. Clin Nucl Med 2003;28:986-987.

Screening for supra-additive effects of cytotoxic drugs and gamma irradiation in an in vitro model for Hepatocellular Carcinoma. B. Lambert, L. De Ridder, G. Slegers, V. de Gelder, R.A. Dierckx, H. Thierens. Can J Physiol Pharmacol 2004;82:146-152.

Renal toxicity after radionuclide therapy. B. Lambert, M. Cybulla, S.M. Weiner, C. Van de Wiele, H. Ham, R.A. Dierckx, A. Otte. Radiat Res 2004;161:607-611.

Bone marrow dosimetry and safety of high 131I activities given after recombinant human thyroid-stimulating hormone to treat metastatic differentiated thyroid cancer. B. de Keizer, A. Hoekstra, M.W. Konijnenberg, F. de Vos, B. Lambert, P.P. van Rijk, C.J. Lips, J.M. de Klerk. J Nucl Med 2004;45:1549-1554.

^{188}Re-HDD/Lipiodol therapy for hepatocellular carcinoma: a phase I clinical trial. B. Lambert, K. Bacher, L. Defreyne, F. Gemmel, H. Van Vlierberghe, J.M. Jeong,

R.A. Dierckx, C. Van de Wiele, H. Thierens, F. De Vos. J Nucl Med 2005;46:60-66.

Radiolabeled Lipiodol therapy for hepatocellular carcinoma in patients awaiting liver transplantation: pathology of the explant livers and clinical outcome. B. Lambert, M. Praet, P. Vanlangenhove, R. Troisi, B. de Hemptinne, Gemmel F, H. Van Vlierberghe, C. Van de Wiele. Cancer Biother Radiopharm 2005;20:209-214.

^{188}Re-HDD/Lipiodol for treatment of hepatocellular carcinoma: a feasibility study in patients with advanced cirrhosis. B. Lambert, K. Bacher, K. De Keukeleire, P. Smeets, I. Colle, J.M. Jeong, R. Troisi, R.A. Dierckx, H. Thierens, F. De Vos, C. Van de Wiele. J Nucl Med 2005;46:1326-1332.

^{188}Re-HDD/Lipiodol therapy for hepatocellular carcinoma: an activity escalation study. B. Lambert, K. Bacher, L. Defreyne, H. Van Vlierberghe, J.M. Jeong, R.F. Wang, J. Van Meerbeeck, P. Smeets, R. Troisi, H. Thierens, F. De Vos, C. Van de Wiele. Eur J Nucl Med Mol Imaging. Accepted for publication.

Treatment of hepatocellular carcinoma by means of radiopharmaceuticals. B. Lambert, C. Van de Wiele. Eur J Nucl Med Mol Imaging. In press.

Clinical applications of Rhenium-188 labelled radiopharmaceuticals for radionuclide therapy. B. Lambert, J.M.H. de Klerk. Nucl Med Commun. Accepted for publication.

Publications in non-peer reviewed national journals

Iood-131 MIBG therapie bij neuro-endocriene tumoren: huidige stand van zaken en indicaties. B. Lambert, B. Brans, M. Monsieurs, G. Laureys, R.Rubens, S. Van Belle, R.A. Dierckx. Tijdschrift voor Geneeskunde 2002,58:1098-1106.

Lokoregionale I-131 lipiodol therapie bij de behandeling van het hepatocellulair carcinoom. F. De Winter, B. Brans, B. Lambert, F. Gemmel, K. Van Laere, L. Defreyne, H. Van Vlierberghe, B. de Hemptinne, K. Bacher, R. A. Dierckx. Tijdschrift voor Geneeskunde 2003,59:532-541.

Communications at international symposia

The mutagenic potential of 131-I-Lipiodol therapy and combined 131-I-Lipiodol-cisplatinum therapy in HCC patients. K. De Ruyck, A. Vral, B. Lambert, R. Dierckx, H. Thierens. 32nd Annual Meetings of European Environmental Mutagen Society. Warsaw, September 3-7, 2002. Poster.

Radiolabeled MIBG and octreotide therapy: Competing or complementary modalities. B. Brans, B. Lambert, E. Entok, A. Kolindou, S. Van Belle, RA Dierckx. 5th International Congress of Nuclear Oncology, Kuçadasi, Turkey, 1-5 May 2002. Poster.

18FDG-PET versus High Dose 67Gallium Scintigraphy for restaging and treatment follow-up of lymphoma patients. B. Van Den Bossche, B. Lambert, F. De Winter, A. Kolindou, RA. Dierckx, L. Noens, C. Van de Wiele. 5th International Congress of Nuclear Oncology. Kuçadasi, Turkey, 1-5 may 2002. Poster.

Détection des effets supra-additives entre la chimiothérapie et l' irradiation externe dans un modèle in vitro du carcinome hépatocellulaire. B. Lambert, R.A. Dierckx, G. Slegers, L. De Ridder, H. Thierens. 6ième Congrès International de Radiobiologie. Batz-sur-Mer, France, June, 30- July, 4 2003. Poster.

Comparison of the cytotoxic effect of 131-I-Lipiodol therapy and 188-Re-Lipiodol therapy in HCC patients . K. De Ruyck, A. Vral, B. Lambert, R.A. Dierckx, H. Thierens. European Association Nuclear Medicine, Annual Congress, Amsterdam, August 23-27, 2003. Poster.

Patient dosimetry after 188Re-Lipiodol therapy. K. Bacher, B. Lambert, F. De Vos, F. Gemmel, C. Van de Wiele, L. Defreyne, J.M. Jeong, G. Slegers, R.A. Dierckx, H. Thierens. European Association Nuclear Medicine, Annual Congress, Amsterdam, August 23-27, 2003. Oral presentation.

Rhenium-188-Lipiodol for locoregional treatment of hepatocellular carcinoma: a phase I study. B. Lambert, K. Bacher, F. Gemmel, L. Defreyne, J.M. Jeong, H. Thierens, G. Slegers, C. Van de Wiele, R.A. Dierckx, F. De Vos. European Association Nuclear Medicine, Annual Congress, Amsterdam, August 23-27, 2003. Oral presentation.

131I-Lipiodol therapy for Hepatocellular Carcinoma in patients awaiting liver transplantation. B. Lambert, M. Praet, L. Defreyne, R. Troisi, B. de Hemptinne, H. Van Vlierberghe, R.A. Dierckx, C. Van de Wiele. European Association Nuclear Medicine, Annual Congress, Helsinki, Finland, September 4-8, 2004. Oral presentation.

Comparison of real-life radiation burden in relatives of patients treated with I131-Lipiodol or 188-Re-HDD/Lipiodol. K. Bacher, B. Lambert, F. De Vos, C. Van de Wiele, R.A. Dierckx, H. Thierens. European Association Nuclear Medicine, Annual Congress, Helsinki, Finland, September 4-8, 2004. Oral presentation.

Development, pharmaceutical validation and radioprotection properties of a new concept of 188W/188Re-generator. F. De Vos, M. De Decker, K. Bacher, B. Lambert, H. Thierens, G. slegers, R.A. Dierckx. European Association Nuclear Medicine, Annual Congress, Helsinki, Finland, September 4-8, 2004. Poster.

Usefulness of FDG PET in intrahepatic and perihilar cholangiocarcinoma. E. Vranken, B. Lambert, P. Smeets, H. Van Vlierberghe, R.A. Dierckx, C. Van de Wiele. European Association Nuclear Medicine, Annual Congress, Helsinki, Finland, September 4-8, 2004. Poster.

Rhenium-188-HDD-Lipiodol treatment of hepatocellular carcinoma: A feasibility study in patients with advanced cirrhosis. B. Lambert, K. Bacher, K. De Keukeleire, I. Colle, J.M. Jeong, R. Troisi, R.A. Dierckx, H. Thierens, F. De Vos, C. Van de Wiele. Society of Nuclear Medicine, Annual Congress, Toronto, Canada, June 18-22, 2005. Oral presentation accepted.

Rhenium-188-HDD/Lipiodol therapy for hepatocellular carcinoma: an activity escalation study. B. Lambert, F. De Vos, K. Bacher, L. Defreyne, H. Van Vlierberghe, J.M. Jeong, R.F. Wang, J. Van Meerbeeck, P. Smeets, H. Thierens, C. Van de Wiele. Society of Nuclear Medicine, Annual Congress, Toronto, Canada, June 18-22, 2005. Poster.

Assessment of supra-additive effects of cytotoxic drugs and high and low dose rate irradiation in an in vitro model for hepatocellular carcinoma. B. Lambert, V. de Gelder, C. Van de Wiele, H. Thierens. European Association Nuclear Medicine, Annual Congress, October 15-19, 2005. Oral presentation accepted.

Communications at national symposia

Radiosensitizers for hepatocellular carcinoma. B. Lambert, K. De Ruyck. Belgian Environmental Mutagen Society Brussels, October 3, 2003. Oral presentation.

Radionuclidentherapie bij het hepatocellulair carcinoom. B. Lambert. 3de symposium Oncologie In Vlaanderen. Bornem, March 29, 2003. Oral presentation.

Rhenium-188-Lipiodol for locoregional treatment of hepatocellular carcinoma: a phase I study. B. Lambert, K. Bacher, F. Gemmel, L. Defreyne, J.M. Jeong, H. Thierens, G. Slegers, C. Van de Wiele, R.A. Dierckx, F. De Vos. Belgisch Genootschap Nucleaire Geneeskunde, Knokke May 25th, 2003. Poster.

Comparison of the cytotoxic effect of 131I-Lipiodol therapy and 188Re-Lipiodol therapy in HCC patients. K. De Ruyck, A. Vral, B. Lambert, R.A. Dierckx, H. Thierens. Belgisch Genootschap Nucleaire Geneeskunde, Knokke May 25th, 2003. Poster.

Rhenium-188-HDD/Lipiodol Therapy for Hepatocellular Carcinoma: An Activity Escalation Study. B. Lambert, F. De Vos, E. Vrancken, K. Bacher, L. Defreyne, H. Van Vlierberghe, J.M. Jeong, J. Van Meerbeeck, P. Smeets, H. Thierens, C. Van de Wiele. Belgisch Genootschap Nucleaire Geneeskunde, Knokke May 21st, 2005. Poster.

Rhenium-188-HDD/Lipiodol Treatment of Hepatocellular Carcinoma: A Feasibility Study in Patients with Advanced Cirrhosis. B. Lambert, E. Vrancken, K. Bacher, K. De Keukeleire, I. Colle, J.M. Jeong, R. Troisi, H. Thierens, F. De

Vos, C. Van de Wiele. Belgisch Genootschap Nucleaire Geneeskunde, Knokke May 21st, 2005. Poster.

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Einde 2002 is de eerste klinische studie met Rhenium-188 gemerkt Lipiodol van start gegaan in ons ziekenhuis. Helaas heeft Frederic dit niet kunnen meemaken. Met zijn scherpe en uitgesproken creatieve geest, zou hij ongetwijfeld een mooie stempel op dit project gedrukt hebben. Ir. Klaus Bacher en Dr. Filip Gemmel zou ik willen bedanken om in de moeilijke periode die daarop volgde, steeds beschikbaar te zijn, met raad en daad. Sinds 2004 nam het klinische werk een snelle vlucht, en ik ben de collega's van de hospitalisatie alsook van de polikliniek Nucleaire Geneeskunde erkentelijk voor de talloze malen dat ze een handje toegestoken hebben. In het bijzonder Dr. Evelyn Vranken, Dr. Ingeborg Goethals, Dr. Olivier De Winter, Dhr. Norbert Hamerlynck, Dhr. Michel Ravier en Dhr. Andy Eeckhout maakten het mogelijk het klinische werk te combineren met het labo-werk op de dienst Medische

Fysica. De inbreng van Dr. Bieke Van Den Bossche -compagnon de route sinds 4 jaar en hopelijk nog veel langer- was dan weer erg nuttig bij de Italiaanse patiënten. Apr. Mario De Decker dank ik voor de vele keren dat hij tijd maakte voor de bereiding van het Rhenium-188 gemerkte Lipiodol.

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Het hele team van de afdeling Interventionele Radiologie beschikt over een mix van vakmanschap, hulpvaardigheid en betrokkenheid bij de individuele patiënt, die de samenwerking erg aangenaam maakte. Hun professionaliteit heeft in belangrijke mate bijgedragen tot het slagen van dit onderzoeksproject. Dr. Katrien De Keukeleire, Dr. Peter Vanlangenhove en Dr. Luc Defreyne ben ik dankbaar voor de talloze keren dat ze de tijd namen om me wegwijs te maken in de overvloed van beelden (toch naar de normen van een nuclearist).

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Ik werd op plezierige wijze ingeleid in de diverse labo-technieken door Lic. Myriam Monsieurs, Apr. Katia Vandenbulcke, Ir. Bart Lucas, Ir. Kim De Ruyck, Mevr. Virginie de Gelder, Dhr. Dirk Van Raemdonck en Dhr. Dirk Terriere. Virginie was gedurende de 2 laatste jaren de rots in de branding wat het labo-werk betreft. Wanneer zij zich over iets ontfermt, doet zij dat steeds met de haar kenmerkende drang naar perfectie en op uiterst charmante wijze. Ik had het geluk dat zij zich over de sferoïden ontfermde.

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The great wall of China, February 19th 2003, working visit First Peking University.
From left to right: Bieke Lambert, Luc Defreyne, Filip De Vos, Klaus Bacher.

This work would not have been possible without the efforts and scientific know-how of Dr. Luc Defreyne (Interventional Radiology), our radiopharmacist Filip De Vos and our physicist Klaus Bacher.

We have gone a long way together. The road was hilly, but the landscape was beautiful and the company was good. I hope we aren't even half way yet.

Cover illustration: papaver rhoedas; back: hibiscus syriacus, national flower of Korea