CLINICAL PROTOCOL

Date of Version: 1/16/2012

Protocol Title: An open-label, single institution phase II study using radioactive yttrium^{90} microsphere (SIR-Spheres® microspheres) in uveal melanoma patients with hepatic metastasis

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Study Medication: Radioactive yttrium^{90} microsphere (SIR-Spheres®)

IRB Number:

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PROTOCOL SUMMARY

Protocol Number:

Protocol Title: An open-label, single institution phase II study using radioactive yttrium^{90} microsphere (SIR-Spheres®) in uveal melanoma patients with hepatic metastasis

Study Objectives:

Primary objectives

(1) Investigate the potential efficacy (clinical response) of radioactive yttrium^{90} microsphere in hepatic metastasis from uveal melanoma.

(2) Investigate the safety of radioactive yttrium^{90} microsphere in patients with metastatic uveal melanoma.
Investigate correlation between molecular characteristics of metastatic uveal melanoma and treatment outcomes.

Secondary objectives:

Investigate overall survival, progression free survival, and duration of response

Study Population: Patients with stage IV uveal melanoma with hepatic metastasis

Study Design: Single institution phase II study.

Investigational Product: Radioactive yttrium^{90} microsphere (SIR-Spheres® microspheres)

Dosage Form: Liquid suspension

Route of Administration:	Intrahepatic arterial infusion
Dosage and Treatment:	Up to 3.0 Giga-Becquerels (GBq) for two treatments
Endpoints:	**Response Rate:** 60% stabilization rate (CR+ PR+SD) warrants future study. **Adverse Events:** Treatment-emergent and related serious adverse events, and Grade 3 and 4 laboratory abnormalities for safety assessments
Other Parameters:	Overall survival (OS), progression free survival (PFS) and duration of response in the liver
Duration of Subject Participation in Study:	Maximum of 2 years after the last radiosphere treatment or death, or study closure
Duration of Follow-up:	Safety data will be collected for three months after the last radiosphere treatment, or initiation of a different type of locoregional or systemic treatment for progression of disease, or death, whichever comes first. Survival information will be collected via phone or visit on a quarterly basis up to 2 years after the last radiosphere treatment or death.
Number of Subjects: Required	48

SIR-Spheres Microspheres Clinical Treatment Plan

Events	Screening Evaluation	Enrollement	Tumor biopsy	Flow Study	Pretreatment assessment	Initial Treatment	Post-Treatment	Second Treatment (*5)	Post-Treatment (*5)	1-month Visit	3-month Visit	Every 3 month follow-up	Exit visit (*6)
TIME	Within 4 weeks prior to enrollment	Within 6 weeks prior to treatment	Within 4 weeks prior to treatment	Within 4 weeks prior to treatment	Within 4 weeks prior to treatment	Day 0	Every 2 weeks	3-5 weeks after Initial Treatment	Every 2 weeks	1 month after the last treatment	3 months after the last treatment	6, 9, 12, 15, 18, 21, 24 months after the last treatment	
Informed consent	X												
Demographics	X												
Medical history	X				X(*4)			X		X	X	X	X
Physical exam	X				X(*4)			X		X	X	X	X
Performance status	X				X(*4)			X		X	X	X	X
Concomitant medications	X				X(*4)			X		X	X	X	X
Dose calculation					X								
Order SIR-Spheres micorspheres					X								
CBC, differential, platelets	X				X(*4)	X	X	X	X	X	X	X	X
PT, PTT, and INR	X				X			X					
Serum biochemistry (*1)	X				X(*4)	X	X	X	X	X	X	X	X
LDH	X				X(*4)	X	X	X	X	X	X	X	X
Pregnancy test (*2)	X				X(*4)								
Study blood drawing						X(*3)				X(*3)	X(*3)		
Liver tumor biopsy			X										
CT scan of chest, abdomen and pelvis	X				X(*4)					X	X	X	X
MRI of abdomen	X				X(*4)						X	X	X
PET scan					X						X	X	X
Volumetric measurement					X								
Hepatic angiogram				X		X		X					
Tc-99 MAA study				X									
Treatment with SIR-Spheres microspheres						X		X					
Toxicities & adverse events					X(*4)	X	X	X	X	X	X	X	X

*1: Comprehensive metabolic panel including total bilirubin, SGOT (AST), SGPT (ALT), alkaline phosphatase, albumin, creatinine, BUN, glucose, calcium

*2: For females of childbearing potential.

*3: 100 ml blood for peripheral blood mononuclear cells, and 10 ml of blood for serum collection.

*4: Tests can be skipped if screening evaluation is performed within 4 weeks prior to scheduled treatment.

*5: If necessary.

*6: Exit visit can be skipped if follow-up visit is performed within 4 weeks.

1.0Background

1.1 Uveal Melanoma

Uveal melanoma is the most common adult primary intraocular malignant tumor (1, 2). The incidence of uveal melanoma is 4.3 cases per million population, with a slightly higher rate in males (4.9 cases per million) when compared with females (3.7 cases per million). Uveal melanoma is more commonly seen in an older age group, with a progressively rising age-specific incidence rate that peaks at the age of 70 years (24.5 cases per million in males and 17.8 cases per million in females) (3).

Up to 50% of patients with posterior uveal melanoma subsequently develop systemic metastasis after the initial diagnosis and treatment (4), however, clinically evident metastatic disease at the time of initial presentation is uncommon, indicating that there is early subclinical metastasis in most cases (5). The liver is the predominant organ involved in 70% to 90% of cases with metastatic uveal melanoma (6-8) and tends to be the first manifestation of metastatic disease (8, 9). Lungs (20%), bone (16%), and skin (12%) are other sites that may be affected (9). The metastatic pattern of uveal melanoma is often distinct from that of the cutaneous form of the disease. Approximately 90% of uveal melanoma patients die with liver metastases (7). Despite achieving great accuracy in correctly diagnosing uveal melanoma in the United States (2), mortality owing to this tumor has remained unchanged over the past 3 decades (5). In general, the survival with metastatic uveal melanoma is poor, with a median survival of less than 6 months (6, 8).

Many cytogenetic investigations of primary uveal melanoma have revealed that the majority of choroidal and ciliary body melanomas are characterized by non-random alternations in chromosomes 1, 3, 6 and 8 (10). Monosomy 3 (either true or functional) and trisomy 8, partial duplication of 8q, or iso-chromosome 8q are the most frequent karyotypic abnormalities present in approximately 50% of cases (10). Recent investigation has indicated that monosomy 3 is a significant predictor of poor prognosis (11). Approximately 70% of patients with monosomy 3 in their primary tumor have died of metastases within 4 years after the initial diagnosis; whereas tumors with normal chromosome 3 status (disomy 3) rarely gave rise to metastatic disease (11). Existence of chromosome 3 abnormality in hepatic metastasis might be correlated to the outcome

of trans-arterial embolization of metastases (12).

1.2 Systemic Treatment for Metastatic Uveal Melanoma

Systemic chemotherapy has failed to show significant efficacy against metastatic uveal melanoma. Retrospective reviews from the M. D. Anderson Cancer Center and ECOG indicated that the response rates to chemotherapeutic drugs commonly used for the treatment of metastatic cutaneous/mucosal melanoma rarely induced responses in patients with uveal melanoma, especially when the liver was involved with metastatic disease (9, 13). Dacarbazine-based chemotherapies that have been used for the treatment of metastatic cutaneous melanoma are ineffective in the treatment of metastatic uveal melanoma (9, 13, 14).

Pyrhonen et al. reported superior results in a pilot study using the combination of bleomycin, Oncovin, lomustine, DTIC (BOLD) plus interferon in a small group of patients with metastatic uveal melanoma (15). This combination has been evaluated in several other clinical trials and was reported to give markedly variable response rates (15-18). The average response rate is 14%, hardly an improvement over other regimens. Neurotoxicity was observed in 13% of the patients. Severe and unpredictable pulmonary toxicity was observed occasionally (16).

The combinations of treosulfan with gemcitabine or cytosine arabinoside were shown to be active in over 70% of uveal melanoma cells tested with *ex vivo* sensitivity test (19). Based on these results, clinical trials using the treosulfan-gemcitabine combination were conducted with modest clinical benefit (20).

Dorval et al. failed to discern any objective responses to high dose IL-2 in their study of patients with metastatic uveal melanoma (21).

1.3 Surgical Treatment for Liver Metastases

Since the liver is the first and in many cases the only site of metastases in patients with uveal melanoma, local treatment aimed at controlling liver metastases holds promise in managing this otherwise highly chemo-resistant tumor. Total resection of the solitary metastasis in the liver or other sites (22, 23) offers a distinct survival advantage. We reported protracted survival with surgery for visceral metastases from uveal melanoma. However, only 9% of patients with

metastases were eligible for surgical intervention (22). The longest median survival of 22 months was observed when a complete surgical excision of the hepatic metastasis was followed by intra-arterial hepatic fotemustine or dacarbazine plus cisplatin (24).

1.4 Hepatic Intraarterial Chemotherapy for Liver Metastases

Hepatic intra-arterial chemoinfusion with an agent that has a rapid systemic clearance rate and a high hepatic extraction rate allows maximum local drug exposure. Leyvraz, et al. reported that intrahepatic chemoinfusion of Fotemustine resulted in a response rate of 40% for patients with liver metastases from uveal melanoma (25). Hepatic intra-arterial chemoinfusion with carboplatin was also used for liver metastases from uveal melanoma (26).

Isolated hepatic perfusion (IHP) has been used for liver metastases from uveal melanoma (27). A series of 22 patients with metastatic uveal melanoma confined to liver was treated at the National Cancer Institute using IHP with melphalan and TNF-α (n=11) or melphalan alone (n=11) (27). There was one treatment-related death. Among the 21 evaluable patients, there were two radiographic complete responses (9.5%) and 11 partial responses (52%). The overall median duration of response was 9 months (range 5-50 months). Recently, a special catheter system has been developed to isolate the hepatic circulation from the systemic circulation. This allows multiple cycles of chemotherapy to be delivered directly to the liver without a major operation (peripheral hepatic perfusion, PHP). A randomized phase III trial comparing PHP and the best available treatment is ongoing.

1.5 Chemoembolization of Liver Metastases

Mavligit et al. reported that hepatic artery chemoembolization with cisplatin and polyvinyl sponge achieved a 46% response rate with a median survival of 11 months (28). We subsequently conducted a phase II trial of hepatic artery chemoembolization using 1,3-bis (2-chloroethyl)-1-nitrosourea (BCNU) in combination with ethiodized oil followed by infusion of a transiently occlusive agent, gelatin sponge particles (29). A total of 29 patients were treated with chemoembolization with BCNU. Among 23 evaluable patients, one patient achieved complete response (CR), 3 patients achieved partial response (PR) and 12 patients achieved stable disease (SD). Median overall survival (OS) of the entire intent-to-treat group of patients was 5.1 months (range 0.1-27.6); for patients with CR or PR, 23.5 months (range 7.4-27.6); SD, 8.7 months (range

2.9-14.4); and progressive disease (PD), 3.3 months (range 1.6-5.6). Importantly, while control of hepatic metastases was obtained, progression was seen in extra-hepatic sites such as spine, lung, lymph nodes, and brain in more than two thirds of patients (29).

1.6 Immunoembolization of Liver Metastases

Since local chemotherapies for metastatic uveal melanoma are not uniformly successful and since even successful local chemotherapies frequently fail at extrahepatic sites, a local immunotherapy is theoretically a reasonable approach to control the liver metastases and to potentially induce a systemic antitumor immune response to prevent distant failure. Based on the above scientific rationale, our group developed a novel approach to the treatment of liver malignancy using embolization of the liver metastases with granulocyte-macrophage colony stimulating factor (GM-CSF) (immunoembolization).

In our phase I clinical study, 34 uveal melanoma patients with less than 50% involvement of the liver were treated with immunoembolization of hepatic metastases with various doses of GM-CSF (30). There were no treatment-related deaths or life-threatening events observed up to and including the 2,000 μg dose level of GM-CSF. Among 31 evaluable patients, there were 2 complete responses (CR), 8 partial responses (PR), and 10 cases of stable disease (SD) in their hepatic metastases. The median overall survival (OS) of intent-to-treat patients was 14.4 months. Multivariate analyses indicated that female gender, high doses of GM-CSF (≥ 1,500 μg) and regression of hepatic metastases (CR and PR) were correlated to longer OS (30).

The result of the phase I trial with immunoembolization was also compared to our institutional historical control of patients who were treated with chemoembolization using BCNU (less than 50% involvement of the liver with metastases). Compared with 19 patients who received chemoembolization, 16 patients who received high dose immunoembolization had significantly better OS (20.4 vs. 9.8 months, $P = .005$) and progression free survival in systemic (extra-hepatic) sites (PFS-S) (12.4 vs. 4.8 months, $P = .001$) (31).

1.7 Selective internal radiation therapy (SIRT, radioactive microspheres) for cancer

Selective internal radiation therapy (SIRT) has been used to deliver high dose of radiation to tumor, while minimizing damage to surrounding normal tissues. Various radioactive particles have been used. In this study, we will use Yttrium-90 (^{90}Y-) microspheres (SIR-Spheres®

microspheres).
Due to the size of microsphere (20-60μm), yttrium-90 microspheres (SIR-Spheres®) become trapped within arteries supplying liver tumors exposing them to high dose radiation.

Hypervascular hepatic tumors such as hepatocellular carcinoma obtain their blood supply almost exclusively from the hepatic artery whereas normal liver parenchyma receives 75% of its blood supply from the portal vein. There are three times more arterial vessels supplying hepatic tumors than normal liver parenchyma. Therefore, the microspheres lodge preferentially within the vasculature of liver tumors, with minimal amounts lodging in the normal liver parenchyma and, ideally, no amounts distributing to other organs, particularly to the lung.

Over 14,000 people have been treated with SIR-Spheres® microspheres at more than 200 locations in >25 countries across the globe. SIR-Spheres® microspheresare currently being used to treat primary and secondary tumors of the liver such as hepatocellular carcinoma and colorectal, breast and neuroendocrine tumors, respectively. There are significant numbers of reports in the literature supporting the efficacy and safety of the use of yttrium-90 for treatment of such tumors. The largest treating countries are the USA, Australia, Germany, Spain, Italy, New Zealand, and Hong Kong, with treatment experience rapidly growing in other EU countries, Turkey and other countries. Treatment has been predominantly for liver metastases derived from colorectal cancer and other solid tumors in western countries and for hepatocellular carcinoma in the Asian countries and Southern Europe.

1.8 SIRT for metastatic uveal melanoma

Radioactive plaque and proton beam radiation have been used for treatment of primary uveal melanoma. Although melanoma cells are generally resistant to radiation, treatment of metastatic uveal melanoma with high-dose local radiation might have therapeutic benefits. In our institution, a total of 12 uveal melanoma patients, five men and 7 women, ages 48-81 (median 65) with hepatic metastases were treated with SIRT due to tumor progression after trans-arterial embolization (n=9), complications of trans-arterial embolization (n=1) or patient preference (n=2) (32). Patients had both hepatic lobes treated on separate occasions (n=7), one lobe (n=3) or whole liver (n=2) therapy. Treatment dose per patient was reduced by 25% due to prior trans-arterial embolization procedures. The mean total SIRT dose delivered was 1.0 GBq (0.62-1.47 GBq). Five patients had

an increase in hepatic enzymes (Grade1-4) at 1-month follow-up. No procedure related deaths or serious adverse events were experienced. Best tumor response was as follows: CR (n=0), PR (n=0), SD (n=8), PD (n=4), with a median follow-up of 7.8 months (1.0-16.0). The median time to liver progression was 7.0 months (1.0-15.5). Nine of the 12 patients, including 2 patients with >25% pretreatment tumor burden, died due to progression of liver disease (n=6), extrahepatic disease (n=1) or both (n=2). Post-SIRT, median overall patient survival was 10.8 months (1.0-19.0). Three patients (ECOG 0 performance status) are still alive at 13.5-19.0 months (median 16.2 months) following SIRT. Although this is a small retrospective study at a single institution conducted in heavily pre-treated patients, the potential efficacy of radiosphere treatment in metastatic uveal melanoma was suggested. Our observation is supported by a report from other institutions (33) and warrants further investigation in a prospective clinical study. In this clinical trial, we will prospectively investigate the safety and efficacy of SIRT using yttrium-90 SIR-Spheres® microspheres in uveal melanoma patients with hepatic metastases. Patients will be stratified based on their history of previous transarterial embolization of the hepatic artery. The safety and efficacy of treatments will be separately analyzed in these two patient cohorts.

2.0 Study Objectives and Endpoints

2.1 Study Objectives

2.11 Primary Objectives

(1) Investigate the potential efficacy (clinical response) of radioactive yttrium^{90} microspheres in hepatic metastasis from uveal melanoma.

(2) Investigate the safety of radioactive yttrium^{90} microsphere in patients with metastatic uveal melanoma.

2.12 Secondary Objectives

(1) Survivals: Investigate overall survival (OS), progression free survival without progression of hepatic metastasis (PFS-L), and duration of response.

(2) Correlation between molecular characteristics of metastatic uveal melanoma and treatment outcomes.

2.2 Study Endpoints

(1) Response Rate: 60% stabilization rate (CR+ PR+SD) warrants future study.

(2) Adverse Events: Treatment-emergent and related serious adverse events, and Grade 3 and 4 laboratory abnormalities for safety assessments.

2.3 Other Parameters

Overall survival (OS), progression free survival (PFS) and duration of response

3.0 Study Design

This is an open-label, uncontrolled single institution phase II study for metastatic uveal melanoma. Uveal melanoma patients who received one or less prior trans-arterial embolization treatment of hepatic metastasis are eligible. Patients will be stratified into two groups: Group A, no prior intra-hepatic arterial treatment, n=24; Group B, one prior hepatic trans-arterial embolization treatment, n=24. They will be treated with intra-hepatic arterial infusion of Yttrium-90 radioactive microspheres (SIR-Spheres® microspheres).

Within 4 weeks prior to radiosphere treatment, patients undergo the pre-assessment angiogram and technetium-99m-labelled macroaggregated albumin (^{99m}Tc -MAA) nuclear scan to block the collateral flow to non-target organs and to calculate the shunting rate to the lung. Once patients meet the eligibility criteria of the study, the radiosphere treatment will be given. The Yttrium-90 radioactive microsphere treatment generally consists of two sequential uni-lobar treatments, approximately 4 weeks (3 to 5 weeks) apart. In selected patients, if clinically feasible, a biopsy of hepatic metastasis will be obtained prior to radiosphere treatment to investigate the correlation between efficacy of treatments and molecular characteristics of metastatic uveal melanoma.

The side effects of Yttrium-90 radioactive microspheres will be monitored every 2 weeks for one month following each treatment and then every month for three months after the last radiosphere treatment. The efficacy of radiosphere treatment will be evaluated every 3 months from the last treatment for 2 years until disease progression or death.

If patients experience grade 3 toxicity after the first treatment with Yttrium-90 radioactive microspheres, the second radiosphere treatment will be held until the resolution of toxicity to grade 1 or less or for a maximum of 6 weeks. The dose of the second radiosphere treatment will be decreased by 50% for liver-related grade 3 toxicity. A dose reduction will not be considered for

grade 3 GI toxicity unless the next treatment is repeated to the same hepatic lobe. The study treatment will be discontinued for grade 4 toxicity or if patients do not recover from the grade 3 toxicity to at least a grade 1 within 6 weeks.

The study will require two years of accrual with an additional two years of follow-up for survival analysis.

4.0 Selection of Patients

4.1 Inclusion criteria

4.11 Histologically confirmed metastatic uveal melanoma in the liver. Patients must have at least one untreated, or progressed liver metastasis that is ≥ 10 mm in longest diameter by spiral CT scan or MRI. The total volume of the tumors must be less than 50% of the liver volume.

4.12 Either no previous trans-hepatic arterial treatment or progressive hepatic metastasis after prior regional treatment with trans-arterial embolization. Embolization of hepatic artery with different types of medication will be considered to be one regional treatment.

4.13 Willingness and ability to give informed consent.

4.14 ECOG performance status of 0, or 1.

4.14 Adequate renal and bone marrow functions:
Serum creatinine ≤ 2.0 mg/dl, granulocyte count ≥ 1000/mm^3, and platelet count ≥ 100,000/mm^3.

4.15 Adequate liver function: total bilirubin <1.6 mg/ml and albumin > 3.0 g/dl,

4.2 Exclusion criteria

4.201 Failure to meet any of the criteria set forth in section 4.1.

4.202 Significant shunting to the lung (>20%) identified on MAA scan.

4.203 Unsuccessful closure of major collateral blood flows from the hepatic artery to non-targeted organs such as the GI tract. Small collaterals that are considered to be safe for Sir-Sphere® treatment by interventional radiologists and radiation oncologists are eligible.

4.204 A solitary liver metastasis that is amenable to surgical removal for potential cure.

4.205 Presence of symptomatic liver failure including ascites and hepatic encephalopathy.

4.206 Presence of life-limiting extra-hepatic metastasis that requires a systemic treatment within 3 months. Patients with extra-hepatic metastasis that can be controlled with a loco-regional treatment are eligible.

4.207 Presence of untreated brain metastases. If patients have had previous treatment for brain metastasis, an MRI or CT scan of the brain must confirm the stabilization of the brain metastasis for more than 4 weeks.

4.208 Occlusion of the main portal vein, or inadequate collateral flow around an occluded branch of the portal vein as determined by angiography.

4.209 Presence of uncontrolled hypertension or congestive heart failure, or acute myocardial infarction within 6 months of entry.

4.210 Presence of any other medical complications that imply a survival of less than six months.

4.211 Uncontrolled severe bleeding tendency or active GI bleeding.

4.212 Significant allergic reaction to iodinated contrast.

4.213 Systemic chemotherapy within 2 weeks prior to study entry (signing consent form).

4.214 Previous radiation treatment that includes the liver in the main radiation field.

4.215 Pregnancy or breast-feeding women.

4.216 Biliary obstruction, biliary stent, or prior biliary surgery including sphincterotomy but excluding cholecystectomy.

4.217 Previous treatment with isolated hepatic perfusion

4.218 Children under the age of 18

5.0 Treatment Schedule

5.1 Pre-study Evaluation

MRI of the abdomen (with and without gadolinium) and CT of the chest, abdomen and pelvis (with and without intravenous contrast). Both imaging exams should be performed prior to enrolling patients to evaluate for extrahepatic disease and tumor burden within the liver. Comprehensive examination including history, physical examination, and laboratory tests to confirm eligibility of patients for the study will also be done (Schedule table). These tests should be done within 4 weeks prior to enrollment of patients to confirm their eligibility. Once their eligibility is confirmed, flow study and radiosphere treatment will be scheduled.

5.2 Flow study (therapy-planning angiography) and pretreatment assessment

Within 4 weeks of radiosphere treatment, therapy planning arteriography of the superior mesenteric and celiac arteries will be performed.

The major purpose of the flow study is as follows:

1) Confirm the ability to selectively catheterize the hepatic arterial vasculature.

2) Assess the flow characteristics in the hepatic arteries.

3) Determine the hepatic arterial supply to the tumor, i.e., right, left or both hepatic arteries.

4) Determine the influence of hepatic arterial anatomy relative to the intrahepatic distribution of tumor on the ability to treat the diseased portion of the liver as a single unilobar treatment, or two sequential unilobar treatments, or 2 fractionated whole liver treatments.
5) Confirm the absence of significant, uncorrectable blood shunting from the liver to the gastrointestinal tract or other abdominal organs (e.g., pancreas).
6) Perform a technetium-99m macro-aggregated albumin (^{99m} Tc-MAA) study to assess the presence and degree of lung shunting of the microspheres from the liver.

During the therapy planning angiography, selective arteriography of the proper, right and left hepatic arteries will be obtained in at least two projections (i.e. PA, RAO) to look for extrahepatic arterial blood supply arising from the hepatic arteries. Coil embolization of extrahepatic arteries (i.e. right gastric, gastroduodenal artery) will be performed as deemed necessary by the interventional radiologist performing the study. Following coil embolization of extrahepatic arteries, ^{99m}Tc-MAA particles will be injected into the proper hepatic artery or injected separately into right and left hepatic arteries by splitting the ^{99m}Tc-MAA dose. The injection location of ^{99m}Tc-MAA will also be at the discretion of the interventional radiologist performing the procedure. The patient will be transported to nuclear medicine for a single photon emission CT (SPECT) scan to assess for shunting to the lungs and extrahepatic activity. CT scan of chest, abdomen, and pelvis with and without contrast, MRI of the abdomen with and without gadolinium, and PET scan will be performed as baseline imaging tests for radiosphere treatment. These tests will be done within 4 weeks of radiosphere treatment.
The dose for treatment will be determined by the radiation oncology attending using the MRI and/or CT scan imaging and the results of flow study using the equations described in Section 9. The determined dose will be ordered.

5.3 Biopsy of hepatic metastasis

Within 4 weeks of radiosphere treatment, tumor specimens will be obtained by core biopsy of a representative hepatic metastasis. DNA as well as RNA will be extracted from the core biopsy specimens after confirming diagnosis of metastatic melanoma by histology and will be used for molecular studies including DNA array analysis and genome-wide SNP analysis. If patients have established diagnosis of metastatic uveal melanoma to the liver, they could decline this biopsy.

The tumor biopsy will also be exempt if the tumor tissue specimen can not be obtained by a conventional technique such as US-guided or CT-guided biopsy.

5.4 Radioactive microsphere treatment

Within 4 weeks after the flow study, selective internal radiation therapy (SIRT) using yttrium-90 SIR-Spheres® microspheres (radiosphere treatment) will be performed. Due to multiple, bilobar metastases, both right and left hepatic lobes need to be treated in the majority of patients. The goal of therapy is to treat the tumor bearing liver in 2 sessions. Treatments are preferentially done in a lobar fashion. In the setting of complex anatomy, fractionated whole liver therapy is used as an alternative. In general, the delivery of radioactive microspheres will require two sequential unilobar treatments separated by a period of 4 ± 1 weeks. The lobe to be treated first will be determined by the treating physicians. The radiosphere dose for each hepatic lobe will be determined based on the tumor volume in individual lobes.

In patients whose hepatic artery does not allow two separate sequential unilobar treatments, whole liver treatment via the proper hepatic artery may be performed if no extrahepatic arteries are identified distal to the catheter site intended for radioactive microsphere delivery. The dose will be delivered in two fractionated doses separated by 4 ± 1 weeks (fractionated whole liver).

If a prior hepatectomy has been performed leaving a single lobe for treatment, or if metastatic disease is confined to a single lobe, the entire dose will be delivered in a single session to the affected lobe.

Treatment will be administered on an outpatient basis with overnight, 23-hour observation permitted at the investigator's discretion. Patients will receive the supportive care as described in Section 7.

Approximately 100ml of blood specimens will be obtained for Peripheral Blood Mononuclear Cell (PBMC) collection prior to radiosphere treatment. Additionally, 10 ml of blood specimens will be obtained for serum collection prior to, and 1 hour after radiosphere treatment.

Blood tests including CBC with differential, comprehensive metabolic panel including total

bilirubin, SGOT (AST), SGPT (ALT), LDH, alkaline phosphatase, albumin, creatinine, BUN, glucose, calcium, and LDH will be ordered every 2 weeks for one month, then every month for three months to monitor treatment-related toxicity.

5.5 One-month post-procedure visit

In 4 weeks (± 1 week) after the last radiosphere treatment (usually after the second radiosphere treatment), patients will be evaluated for treatment-related acute toxicity. Patient will also have a 1-month follow-up CT scan of the chest, abdomen and pelvis (with and without contrast) to assess extrahepatic disease.

Approximately 100ml of blood specimens will be obtained for PBMC collection. Additionally, 10 ml of blood specimens will be obtained for serum collection.

5.6 3-month post-procedure visit

Three months (± 2 weeks) after the last radiosphere treatment (usually after the second radiosphere treatment), patients will be evaluated for response to treatment and late toxicity. CT scan of the chest, abdomen and pelvis (with and without contrast); MRI of the abdomen (with and without gadolinium) and PET scan will be obtained.

Approximately 100ml of blood specimens will be obtained for PBMC collection. Additionally, 10 ml of blood specimens will be obtained for serum collection.

5.7 Subsequent follow-up visits

After the 3-month office visit, patients will be followed every three months for the maximum of two years. CT scan of the chest, abdomen and pelvis (with and without contrast); MRI of the abdomen (with and without gadolinium), PET scan, and blood test including CBC with differential, comprehensive metabolic panel including total bilirubin, SGOT (AST), SGPT (ALT), LDH, alkaline phosphatase, albumin, creatinine, BUN, glucose, and calcium will be obtained prior to the individual follow-up visits.

5.8 End of study visit

At the conclusion of two-year follow-up or upon withdrawal of patients from the study, patients will be evaluated by a study physician. CT scan of the chest, abdomen and pelvis (with and without contrast); MRI of the abdomen (with and without gadolinium), PET scan, and blood test including CBC with differential, comprehensive metabolic panel including total bilirubin, SGOT (AST), SGPT (ALT), LDH, alkaline phosphatase, albumin, creatinine, BUN, glucose, and calcium will be ordered. If patients are evaluated with the above tests within 1 month prior to the conclusion of study, this visit will be substituted for the end of study visit.

5.9 Withdrawal of subjects

The following criteria will be used to withdraw patients from the study.

1. Progressive disease (PD) by RECIST criteria or at the end of 2 years following treatment initiation. Patients will be withdrawn from the study if significant extra-hepatic disease develops and systemic therapy is required. In addition, if patients demonstrate significant intra-hepatic tumor progression and require additional percutaneous or catheter-directed therapy, they will be withdrawn from the study.
2. Unacceptable toxicity. This is defined as greater than or equal to grade 4 toxicity.
3. The patient may withdraw from the study at any time for any reason with written notification to the investigator.
4. Investigator's decision that it is in the patient's best interest to come off study.
5. A major protocol violation, or if requested by the sponsor or regulatory agency.
6. The withdrawal of a subject from the trial may also occur if the subject is noncompliant. This will not affect the standard of the patient's future medical care.
7. Death of a subject. **If a subject dies while participating in this study, regardless of the cause, an SAE form must be completed.**

5.10 Follow-up after withdrawal from study

All patients, including patients who discontinue protocol therapy because of toxicity or progressive disease must be followed every three months up to a minimum of 2 years to determine clinical status and to document time to progression and duration of survival. Telephone contact and record review is allowed for this purpose. If patients are removed from study due to grade 4 toxicity, patients must be followed until toxicity level decreases to ≤ grade 1 or considered resolved with sequelae.

For patients who achieved PR, CR, or SD in the liver metastases after radiosphere treatment, tumor measurements should be continued every 3 months for up to 2 years after initiation of protocol therapy or until PD in the liver metastases is confirmed.

6.0 Supportive Care Guidelines

6.1 Post-Treatment Care

Following treatment, the patient will remain under observation according to standard care guidelines for the aftercare of procedures involving femoral artery catheterization. This typically involves at least 6-hour monitoring. After 6 hours of observation, the patient will be sent home when the interventional radiology (IR) attending physician determines that the patient is stable and that there is no risk of bleeding from the catheter site. Patients could be kept over night for further observation at the discretion of IR attending or study physicians. At the time of discharge, patients will be instructed regarding aftercare and will be provided with a 24-hour telephone number that they may use to contact the IR attending or study physician if they develop a problem.

6.2 Pre- and Post-treatment Supportive Therapies

The following supportive therapy will be administered at the discretion of investigator, unless otherwise contraindicated:

(1) Pre- and post-therapy intravenous hydration.

(2) Gastrointestinal prophylaxis to prevent GI inflammation and ulceration: a proton pump inhibitor (e.g. Pantoprazole 40mg orally once daily) continuing for 4 weeks post treatment.

(3) Anti-nausea prophylaxis: e.g., anti-emetics (e.g. Ondansetron 8mg orally or i.v., every 8 hours, PRN) for post-treatment nausea.

(4) Post-embolization syndrome prophylaxis: Provided the patient is not diabetic – and oral steroids are not otherwise contraindicated – a tapering dose of oral corticosteroids (e.g., Methylprednisolone 24mg, 20mg, 16mg, 12mg, 8mg, 4mg orally on days 0, 1, 2, 3, 4 & 5 respectively, or dexamethazone 4 mg, bid for 3 days, then once a day for 3 days) commencing on the day of treatment will be administered.

(5) Pain control: Oral analgesia (e.g., Ketorolac 10mg orally every 6 hours PRN, or oxycodone 5 mg, orally every 4 hours PRN) may be required for 1 week following treatment to relieve pain from radiation injury and embolic effects related to SIR-Spheres® microspheres, and liver capsular pain from tumor edema.

7.0 Evaluation of Response

7.1 Evaluation of clinical response

7.1.1 Definition of Measurable Disease

Measurable disease

Measurable disease is defined by the presence of at least one measurable lesion as defined below and adapted from the RECIST criteria version 1.1 (34). All measurements must be recorded in metric notation. If measurable disease is restricted to a solitary lesion, its malignant nature should previously have been confirmed by cytology/histology.

Since some melanoma metastases contain melanin pigment and since successfully treated liver metastases may develop significant necrosis, evaluation of radiological response after embolization treatments is generally difficult. Therefore, we will obtain CT scan and MRI of the liver, and PET scan of the body for the precise evaluation of radiological responses. However, the same modality will be used for measurement of the liver metastases and assessment of the response. We prefer to use MRI for response assessment due to its superiority in measurement of tumor size and assessment of tumor necrosis compared to traditional spiral CT scans.

The longest diameter (LD) of the largest liver lesion should be ≥ 10 mm by spiral CT scan or MRI in order to become eligible for the study.

Non-measurable disease

All other lesions including small lesions (<10 mm on spiral CT scans or MRI) are considered "non-measurable". The following lesions are also considered as "non-measurable": Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusion, abdominal masses that are not confirmed and followed by imaging techniques, tumors treated by irradiation or by intra-tumor injection therapy.

7.1.2 Baseline Documentation of "Target" and "Non-Target" Lesions

Target Lesions (Indicator Lesions)

The target lesions are defined as measurable lesions that will be used for evaluation of response. For this protocol, target liver lesions require serial measurement of a longest diameter and should be ≥10 mm by spiral CT scan or MRI at the time of study entry.

Target lesions (up to a maximum number of 5 for the liver metastases) must be identified and measured at baseline prior to the initiation of radiosphere treatment. Target lesions must be selected on the basis of their size (LD) and suitability for repetitive measurements.

Non-Target Lesions

All other lesions (or sites of melanoma) must be identified as "non-target" and their location and characteristics must be recorded at baseline. During follow-up evaluations, these lesions must be followed as "present" or "absent".

7.1.3 Evaluation of overall response in the liver metastases

Clinical response in the liver metastases will be evaluated 3 months after the last radiosphere treatment using CT scans or MRI of the abdomen. The same modality must be used for serial measurements of target lesions. The sum of the longest diameter (LD) of up to 5 target liver lesions will be used to determine response. The investigators must identify target indicator lesions and measure them within 4 weeks prior to the first radiosphere treatment as baseline. The investigators will then measure the same target lesions 3 months after the last radiosphere treatment.

The sum of the baseline LDs will be compared to the sum of LDs after radiosphere treatments. New lesions will be defined as lesions that appear in the treated lobe(s) of the liver after the baseline evaluation and should be ≥ 10 mm in longest diameter. Development of new lesion(s) in an untreated lobe (area) of the liver **will not** be considered as "Progressive Disease" for evaluation of liver response.

7.1.4 Response criteria for liver metastases

The response of liver metastases will be evaluated by MRI or CT scans using the criteria adapted from the international criteria proposed by the Response Evaluation Criteria in Solid Tumors (RECIST) Committee. The same imaging modality must be used for the serial measurements of

tumor diameter. The response must be confirmed by two consecutive imaging tests performed not less than 4 weeks apart. The definition of response of liver metastases in the treated lobe(s) is shown in Table 1. Evaluation of target and non-target lesions in the treated liver lobe(s) to determine response of liver metastases is shown in Table 2.

In terms of PET scan assessment, up to 6 metabolically active metastases with SUV of 2 or greater will be recorded prior to the radiosphere treatment. If more than 50% of these lesions show SUV of 2 or less at 3 month PET assessment, the radiosphere treatment will be considered to be "effective" (35).

Table 1. The definition of response of liver metastases in the treated lobe(s)

Complete Response (CR)	Disappearance of all target and non-target liver lesions
Partial Response (PR)	$\geq$ 30% decrease in the sum of the longest diameters ("sum LD") relative to baseline sum LD with at least stable non-target liver lesions
Stable Disease (SD)	Absence of change which would qualify as response or progression
Progression (PD)	$\geq$ 20% increase in the sum LD in target liver lesions or unequivocal progression of non-target liver lesions in the treated lobe(s) Appearance of one or more new liver lesions $\geq$10 mm in the treated lobe(s)

To be assigned a status of CR or PR, changes in tumor measurements must be confirmed by the follow-up assessment using the same imaging test at a minimum interval of 4 weeks. The patients who have a CR or PR without confirmatory assessment will be categorized as SD. In the case of SD, follow-up measurements must have met the SD criteria at least once at a minimum interval of 4 weeks. The patients who have a SD without confirmatory assessment will be categorized as PD.

Table 2. Evaluation of target and non-target lesions in the treated liver lobe(s)

Target Liver Lesions	Non-Target Liver Lesions	New Liver Lesions in the treated lobes	Overall Liver Response
CR	CR	No	CR
CR	SD	No	PR
PR	Non-PD	No	PR
SD	Non-PD	No	SD
PD	Any	Yes or No	PD
Any	PD	Yes or No	PD
Any	Any	Yes	PD

7.2 Progression free survival in hepatic metastases (PFS-L)

PFS-L is measured from the start of the treatment to confirmation of progression of hepatic metastasis by either imaging tests or physical examination, or death of patient, whichever comes first.

7.3 Overall survival

Overall survival (OS) is measured from the start of the treatment to patient death. Date and cause of death will be recorded. The cause of death will be categorized as either cancer-related or cancer-unrelated.

7.4 Molecular characteristics of tumor and response to treatments

It has been reported that DNA arrays using tumor specimens could predict the survival of patients with primary uveal melanoma (36). We also reported that presence of monosomy 3 in liver biopsy specimens is correlated to poor prognosis in patients who received embolization of hepatic metastasis (37). We will investigate whether the above observations are also seen in patients who receive radiosphere treatments. We hypothesized that the presence of monosomy 3 in hepatic metastasis is correlated to resistance to radiosphere treatment. Furthermore, correlation between specific gene expression patterns and clinical response will be investigated. We will investigate whether the same gene profile reported in the literature (36) predicts the outcome of radiosphere treatment. Hepatic tumor specimens will be obtained by core biopsy prior to radiosphere treatment. DNA as well as RNA will be extracted from the tumor specimens after confirming diagnosis of metastatic melanoma. RNA specimens will be reverse-transcribed to cDNA and

tested with DNA microarray analysis. Furthermore, extracted DNA specimens will be tested with a genome-wide SNP analysis and the correlation between genomic abnormality such as monosomy 3 and/or excess copies of chromosome 8, and response to radiosphere treatment will be investigated (38). Peripheral blood mononuclear cells will be used as a control for molecular/genetic analysis of tumor specimens.

7.5 Immunological and cytokine response to radiosphere treatment

It has been reported that local liver treatments induce inflammatory cytokines and also change the levels of angiogeneic cytokines (39)(40), which might contributes to a clinical response to the treatment. We have also reported that a patter of productions of IL-6, IL-8, and TNF-alpha is different between uveal melanoma patients who received immunoembolization with GM-CSF for their hepatic metastases and those treated with plain embolization without GM-CSF (41). It is speculated that radiation treatment to established tumors would result in destruction of tumor-induce immunosuppressive microenvironment and might induce an anti-tumor immune response. To investigate this hypothesis, blood specimens will be collected prior to, and after radiosphere treatments and changes in serum cytokines and T cell subpopulation including regulatory T cells and memory T cells will be investigated. We hypothesized that patients with a good clinical response have decrease in circulating regulatory T cells and increase in a subset of memory T cells as seen in immunoembolization studies (unpublished data).

Approximately 100ml of blood specimens will be obtained for PBMC collection prior to radiosphere treatment and then at 1-month and 3-month office visits. Additionally, 10 ml of blood specimens will be obtained for serum collection prior to, and 1 hour after radiosphere treatment, and then at 1-month and 3-month office visits.

8.0 Drug Information

8.1 Product Description

SIR-Spheres® microspheres consist of biocompatible microspheres containing yttrium-90 with a size range of 30 to 40 microns in diameter. Yttrium-90 is a high energy pure beta radiation emitting isotope with no primary gamma emission. The maximum energy of the beta particles is 2.27MeV with a mean of 0.93MeV. The maximum range of these emissions in tissue is 11mm with a mean range of 2.5mm. The half-life of yttrium-90 is 64.1 hours. In use requiring the isotope to decay to

infinity, 94% of the radiation is delivered in 11 days, leaving only background level radiation, which has no therapeutic value. SIR-Spheres® microspheres themselves are a permanent implant. Each device is for single patient use.

SIR-Spheres® microspheres are commercially available from:
Sirtex Medical Inc.
16 Upton Drive, #2-4
Wilmington, MA 01887
Fax: (978) 229-9585
Email: csusa@sirtex.com

8.2 Calibration of Radioactivity

Yttrium-90 is a pure beta emitter with a half-life of 64.1 hours. The activity of SIR-Spheres® microspheres is calibrated to 09:00 hours Sydney time for Australia, which is 18:00 USA Eastern Standard Time for the United States and 23:00 Greenwich Mean Time for Europe and is supplied with an activity of 3 Giga-Becquerels (GBq) ± 10% at time of calibration..
After 14 days, only 2.5% of the original activity remains.

8.3 Biological Properties and Action

Following implantation via a hepatic artery catheter, SIR-Spheres® microspheres become embolized in the microvasculature of liver tumors, where they have a local radiotherapeutic effect. Some limited concurrent damage to healthy hepatic parenchyma is caused by radiation that escapes tumor boundaries and from SIR-Spheres® microspheres that fail to become embedded in tumors. Following decay of the yttrium-90 to stable zirconium-90, the inert microspheres remain implanted in the tumor tissue. The device is not phagocytosed, nor does it dissolve or degrade after implantation. High dose radiation emitted from the device is cytocidal to cells within the range of the radiation. Treatment with SIR-Spheres® microspheres exploits a normal physiological process to selectively target the cancerous tissue. Healthy liver tissue receives the overwhelming majority of its blood supply via the portal vein and much less from the hepatic artery. However, when tumors develop in the liver, the overwhelming majority of tumor blood supply is via branches of the hepatic artery and much less from the portal vein. The consequence of this is that

catheterization and administration of agents via the hepatic artery permits the preferential targeting of therapeutic material to liver tumors.

8.4 Expected Adverse Reactions

Common adverse effects

When the patient is treated with the proper technique, without excessive radiation to any organ, the common adverse events after receiving SIR-Spheres® microspheres are fever, transient decrease in hemoglobin, mild to moderate abnormality of liver function tests – specifically a mild increase in SGOT, alkaline phosphatase and bilirubin, abdominal pain, nausea, vomiting, and diarrhea. The majority of adverse events are grade 1 and 2 toxicity.

Most patients develop a post-operative fever that starts immediately after implantation of SIR-Spheres microspheres® and can last from a few days to a week. The fever does not necessarily indicate sepsis but may be related to the embolic effect of the microspheres and the acute toxic effects on the tumor. If there is any suspicion of bacterial infection, blood culture will be performed and appropriate antibiotics will be given.

Many patients experience nausea that may last up to several weeks and this may occasionally be severe enough to require anti-emetic medication that should be continued until the symptoms subside.

Serious Abdominal Pain

Many patients experience abdominal pain immediately after administration of SIR-Spheres® microspheres and may need pain relief with narcotic analgesia. The pain generally subsides within an hour or so, but patients may require oral analgesia for up to several days.

Immediate, excessive abdominal pain after implantation of SIR-Spheres® microspheres may indicate that microspheres have been inadvertently delivered to other organs such as the pancreas, stomach or duodenum. This will result in acute pancreatitis or peptic or duodenal ulceration. A post-implantation nuclear scan will verify the placement of the microspheres. This is performed with a gamma camera, which will pick up the secondary radiation from the yttrium-90. The introduction of microspheres into the vasculature of the stomach, duodenum or other organs of the gastrointestinal tract can cause chronic pain, ulceration and bleeding. Additional tests such as

serum amylase are also indicated if pancreatitis is suspected. If this were to occur the patient should be treated using best standard practice, including pain relief, and intravenous fluids.

The development of acute peptic ulceration is suggested by the recognized symptoms of ulcer disease and diagnosed by endoscopy. If this were to occur, the patient should be treated using best standard practice, including pain relief, gastric acid blocking drugs and intravenous fluids. Treatment is the same as for any cause of acute peptic ulceration.

Delayed Serious Events

Radiation Pneumonitis

Microsphere shunting to the lungs can cause edema and fibrosis that may not be reversible. Extrahepatic shunting may be identified through the injection of ^{99m}Tc-MAA into the hepatic artery (20, 21). High levels of implanted radiation and/or excessive shunting to the lung may lead to radiation pneumonitis. This may be suspected if patients develop a non-productive cough several days or weeks after the implantation of SIR-Spheres® microspheres and is diagnosed by chest X-ray. Patients should be treated with systemic corticosteroids and supportive care until symptoms have subsided.

Radiation Hepatitis

The use of radiosphere leads to irradiation of both tumor and normal liver parenchyma. As a result, patients with diseases which compromise the functioning of the liver parenchyma or with very small lesions scattered throughout the normal parenchyma may be at greater risk of liver function impairment. Excessive radiation to the normal liver parenchyma may result in radiation hepatitis. This can be difficult to diagnose, and may appear many weeks after the implantation of SIR-Spheres® microspheres. It is suspected if there is unexplained progressive deterioration in liver function. The diagnosis can be confirmed by histological examination of core liver biopsy. If the diagnosis is suspected or proven then patients should be treated with systemic corticosteroids and supportive care until the inflammation settles.

8.5 Exposure in Utero

There are no studies on the safety and effectiveness of SIR-Spheres® microspheres in pregnant women, nursing mothers or children. Women of childbearing age requiring SIR-Spheres®

microspheres should be treated when non-pregnancy can be ascertained. Safety in pregnancy and childhood has not been established and SIR-Spheres® microspheres should not be implanted into these patients.

The patient may emit low levels of radiation for several weeks, therefore care must be taken with pregnant women and children in close proximity to the patient.

9.0 Dose calculation

9.1 Dose calculation methods

The detail method to calculate the dose of SIR-Spheres® microspheres is described in the Appendix 1 (SIR-Spheres User Manual).

There are two methods for calculating the activity of SIR-Spheres® microspheres to implant –**BSA method** and **Partition model**. In this study, two methods will be combined to give the maximum dose to tumors and to avoid serious radiation injury to the normal liver and lung. The final dose for treatment will be determined by radiation physicist and radiation oncologist.
However, the radiation dose to the normal liver parenchyma should not exceed 35 Gray in patients with normal liver and 30 Gray in patients with cirrhosis. The dose to the lung should not exceed 20 Gray. The dose received by the tumor has no upper limit.

9.1.1 Body Surface Area (BSA) Method

(1) Tumor involvement: $TI = \frac{V_T}{V_L}$
(calculated by CT scan)

(2) Body Surface Area: **BSA**(m^2) = $0.20247 \times H^{0.725} \times W^{0.425}$

(3) Total Activity of SIR-Spheres (GBq): **A** = BSA - 0.2 + TI

It should be noted that the calculated activity of yttrium-90 may have to be further reduced if the percentage lung shunting is greater than 10% as demonstrated by the a technetium-99m scan (nuclear medicine break-through scan) (**See Section 9.2, Dose Modification**) .

9.1.2 Partition Model for Calculation of Dose/Activity of SIR-Spheres microspheres

This method involves implanting the highest possible activity to the tumor while maintaining radiation dose to sensitive tissues such as the lung and the normal liver. Therefore this method provides the highest radiation dose to the tumor that is associated with protection of normal tissue from radiation damage.

The following equation is used to calculate the radiation dose received by an organ after SIR-Spheres® microspheres have been delivered to that organ:

(1) Lung shunt fraction: **F**= Total lung counts/(Total lung counts + Total liver counts)

(2) Tumor to normal liver uptake ratio: **R**=Tumor counts/normal liver counts, using a sample region in tumor and normal liver of the same size (If more than one region is sampled, the an average value is calculated)

(3) NOMINAL INFUSED TREATMENT DOSE PER GBq TOTAL ACTIVITY

$$D/A = 49.67\ (1-F)\times\frac{1}{M_{\mathrm{L}}}$$

(4) NOMINAL DOSE TO TUMOR PER GBq TOTAL ACTIVITY

$$D_{\mathrm{tumor}}/A = 49.67\ (1-F)\times\frac{R}{[M_{\mathrm{L}} + M_{\mathrm{T}}\times(R-1)]}$$

(5) NOMINAL DOSE TO LIVER PER GBq TOTAL ACTIVITY

$$D_{\mathrm{liver}}/A = 49.67\ (1-F)\times\frac{1}{[M_{\mathrm{L}} + M_{\mathrm{T}}\times(R-1)]}$$

(6) NOMINAL DOSE TO LUNG PER GBq TOTAL ACTIVITY

(Lung volume is estimated as 1000cc, 1,000g)

$$D_{\mathrm{lung}}/A = 49.67\times F$$

Where:

A = prescribed activity

D_{tumor} = dose to tumor

F = lung shunt fraction

M_L = mass of liver including tumor

M_T = mass of tumor

R = tumor:normal liver uptake ratio

D_{liver} = dose to normal liver

D_{lung} = dose to lung

Note: All activities are in GBq and masses in g (grams). For the purpose of calculating tissue mass, all tissue densities are estimated at 1g/cc.

Tumor and liver volumes can generally be determined using the diagnostic package associated with the CT scanner. The tumor and normal liver volumes in individual hepatic lobes (i.e., right and left lobe) will be calculated separately and the radiosphere dose to each lobe will be decided independently. If dose estimation for individual lobes is not feasible, the radiosphere dose will be split to 70% (right lobe) and 30% (left lobe). If an older scanner is used, the CT scan of the liver is performed using 10 mm slices. The tumor and total liver areas are traced out for each slice of the MRI and/or CT scan. This is traced using a graphics tablet and the total areas multiplied by 10 mm to give the volume of tumor and normal liver.

9.2 Dose Modification

9.2.1 Previous embolization treatment

The calculated protocol dose of SIR-Spheres® microspheres can be modified. If patients have had prior trans-arterial embolization, then the calculated protocol dose will be decreased by 25%. The dose may also be decreased to limit the total radiation dose to the liver to 30 Gy.

9.2.2 Shunting to the lung

The percent lung shunting may alter the activity that can be safely implanted commensurate with an acceptable risk of radiation pneumonitis. The following recommendations apply:

Percent Lung Shunting Activity of SIR-Spheres® microspheres

<10%: Deliver full amount of SIR-Spheres® microspheres
10% to 15%: Reduce amount of SIR-Spheres® microspheres by 20%
15% to 20%: Reduce amount of SIR-Spheres® microspheres by 40%
>20%: Do not give SIR-Spheres® microspheres

The reduction in the activity implanted should be considered in light of the radiation dose that may be received by the tumor. In some patients, a reduction in activity of 20% may ensure the safety of the lung, but no longer provide sufficient radiation to the tumor. This will depend on the bulk of tumor being treated and the tumor to normal ratio of SIR-Spheres® microspheres deposition. This can be determined from the nuclear medicine breakthrough scan, in which the amount of MAA in the liver can be quantified into that in the tumor and that in the normal liver.
The final dose of individual treatment will be determined by a radiation oncologist based on the information describe above.

10.0 Radiation Safety

Personnel involved in any aspect of handling SIR-Spheres® microspheres must be suitably qualified and be appropriately trained to deal specifically with this device. This includes nuclear medicine staff, staff involved in the implantation procedure and in post-implant care of the patient. Such staff requires the support of a radiation safety officer or expert in radiation physics, and licenses for the facility will normally require that such expertise is available to ensure safe use of isotopes within the facility. Complete information will be found in the Radiation section in the Sirtex Medical User Manual (Appendix 1).

Radiation exposure to the treatment team and to either nursing staff or visitors following the implantation is presented in depth in the Sirtex Medical User Manual. The representative exposure levels for the technician or pharmacist preparing a typical patient dose, and for the physician implanting that prepared dose of SIR-Spheres® microspheres are also included in this manual.

11.0 Safety Assessment

11.1 Definitions

11.1.1 Adverse Event (AE)

An AE is any untoward medical occurrence that happens during the specified medical event reporting period regardless of whether or not it is considered related to treatment.

AEs include the following:

- All suspected adverse medication reactions.
- All reactions from medication, overdose, abuse, withdrawal, sensitivity, or toxicity.
- Apparently unrelated illnesses, including the worsening of a preexisting illness (see Preexisting Conditions below).
- Injury or accidents. Note that if a medical condition is known to have caused the injury or accident (*e.g.*, a fall secondary to dizziness), the medical condition (dizziness) and the accident (fall) should be reported as two separate adverse events.
- Abnormalities in physiological testing or physical examination (findings that require clinical intervention or further investigation beyond ordering a repeat [confirmatory] test).
- Laboratory abnormalities that require clinical intervention or further investigation (beyond ordering a repeat [confirmatory] test) unless they are associated with an already reported clinical event.

Diagnostic and therapeutic non-invasive and invasive procedures, such as surgery, should not be reported as adverse events. However, the medical condition for which the procedure was performed should be reported if it meets the definition of an adverse event.

11.1.2 Preexisting Conditions

In this trial, a preexisting condition (*i.e.*, a disorder present before the adverse event reporting) should not be reported as an adverse event unless the condition worsens or episodes increase in frequency during the adverse event reporting period. Recurrence or progression of tumor will not be regarded as an adverse event, but will be recorded for patient follow-up.

11.1.3 Adverse Event Reporting Period

The adverse event reporting period for this trial begins when the patient receives the first radiosphere treatment and ends 3 months after the last radiosphere treatment. All adverse events that occur in trial subjects during the adverse event reporting period specified in the protocol will be reported, whether or not the event is considered treatment-related.
In addition, even after the completion of adverse event reporting period, if investigators consider that patients develop a serious adverse event that would be possibly related to the study treatment, such SAE will be reported.

11.1.4 Seriousness

Each adverse event is to be classified by the investigator as SERIOUS (including life-threatening) or NON-SERIOUS. This classification of the gravity of the event determines the reporting procedures to be followed.
An adverse event that meets one or more of the following criteria/outcomes is classified as SERIOUS:

- Death
- Life-threatening (*i.e.*, immediate risk of death)
- Inpatient hospitalization or prolongation of existing hospitalization
- Persistent or significant disability/incapacity
- Congenital anomaly/birth defect in the offspring of a subject
- An event not included in the list above, but one that jeopardizes the subject or may require intervention to prevent one of the outcome listed above

A NON-SERIOUS event is one that does not meet the criteria described for a serious or life-threatening event.

11.2 Evaluation of Adverse Events

The Common Terminology Criteria for Adverse Events (CTCAE version 4.0) (http://ctep.cancer.gov/protocoldevelopment/electronic_applications/docs/ctcaev4.pdf) will be used to grade the maximum intensity of the event.

The acute toxicity assessment will be performed 1 month after the last radiosphere treatment. The patient will be re-evaluated for late toxicity at 3-month follow-up visit. After completion of the 3-month visit, patients will be followed every three months for 2 years for survival. Follow-up over the telephone will be allowed for this purpose. During this follow-up period, if investigators consider that patients develop a serious adverse event that would be possibly related to the study treatment, such SAE will be reported.

11.3 Attribution of Causality of Adverse Events

One of the investigators will assign an attribution to each AE that occurs. Attribution will be determined according to the criteria specified in CTCAE v4.0, which are summarized below:

5-Definite – The AE is *clearly related* to the treatment.

4-Probable – The AE is *likely related* to the treatment.

3-Possible – The AE *may be related* to the treatment.

2-Unlikely – The AE is *doubtfully related* to the treatment.

1-Unrelated – The AE is *clearly not related* to the treatment.

11.4 Reporting Adverse Events

Any adverse event that is associated with treatment and that is both serious and unexpected will be reported to FDA by telephone or FAX within 7 days. All of the investigators and the Thomas Jefferson University IRB will be notified as well. A follow-up safety report will be submitted within 15 days of initial notification.

In addition, the Sirtex Medical Inc. is to be notified within 24 hours of becoming aware of any serious adverse event.

Medical Director
Sirtex Technology Pty. Ltd.
Email: qa@sirtex.com
Fax: +61 2 9936 1404

AE's will be reported to the Thomas Jefferson University IRB and the Clinical Research Management Office (CRMO) as specified in the TJU Data Safety and Monitoring Plan. **Unexpected fatal adverse events associated with treatment will be reported within 24 hours to the TJU IRB, to the Data Safety Management Board (DSMB) and to Sirtex.** Fatal adverse events not related to treatment will be reported to the TJU IRB and to the DSMB within 5 days.

AE will be reported according the table below.

Unexpected Event		
Grade 1-3 Attribution of Possible, Probable or Definite	**Grade 4** Regardless of Attribution	**Grade 5** **Death**
Report to CRMO via website within 48 hours. Report to TJU IRB within 48 hours. CRMO reports to DSMB immediately following receipt.	Report to CRMO via website and TJU IRB within 48 hours. CRMO reports to DSMB immediately following receipt.	Report to CRMO via website and TJU IRB within 24 hours. Report to Sirtex within 24 hours.

Expected Event		
Grades 1-3	**Grade 4** Regardless of Attribution	**Grade 5** **Death**
Report to CRMO via website within 10 working days. Summary of all adverse events submitted quarterly to DSMB.	Report to CRMO via website and TJU IRB within 48 hours. CRMO reports to DSMB immediately following receipt. Summary of all adverse events submitted quarterly to DSMB.	Report to CRMO via website and TJU IRB within 24 hours. Summary of all adverse events submitted quarterly to DSMB.

11.5 Thomas Jefferson University Data and Safety Monitoring Plan

The study will be conducted in accordance with the Thomas Jefferson University Data and Safety Monitoring Plan. Adverse events will be recorded and graded based on CTCAE v4.0. All adverse events will be reported to the Kimmel Cancer Center Clinical Research Management Office (CRMO) using a password-protected web-site. **Unexpected fatal adverse events associated with radiosphere treatment will be reported within 24 hours to**

the TJU IRB. Fatal adverse events not related to radiosphere treatment will be reported to the TJU IRB and to the DSMB within 5 days. None-fatal events will be reported according to the table in Section 11.4.

11.6 Follow-Up of Adverse Event

All AE's will be followed until they are resolved or the subject's participation in the trials ends. In addition, all SAE's will be followed by one of the investigators at Thomas Jefferson University even after the subject's participation in the trial is completed until they resolve or until one of the investigators assesses them as "chronic" or stable".

12.0 Statistical Considerations

This is a phase II clinical trial to investigate safety and efficacy of radioactive microsphere (*SIR-Spheres® microspheres*). Uveal melanoma patients with progressing hepatic metastases who received no more than one intra-hepatic arterial treatment will be enrolled. Patients will be first stratified into two groups: Group A, no prior intra-hepatic arterial treatment; Group B, one prior intra-hepatic arterial treatment).

A total of 24 uveal melanoma patients will be enrolled into each group. The primary endpoint of this study is the clinical benefit rate (CR+PR+SD). A Simon's two-stage optimal design (alpha=0.05, beta=0.20) will be applied to each group of patients (42, 43). The 60% of clinical benefit rate will be considered effective enough for the future investigation of this treatment. The radiosphere treatment will be considered to be ineffective if clinical benefit rate is 30% or less.

Choice of design is guided by a desire to stop the trial early if the actual stabilization rate is 30% or less. If the stabilization rate is 60% or greater, we would like to have a low probability of failing to conclude effective. Based on the optimal design, we will:

	Look after this number of patients	Stop if number of successes is less than or equal to
Stage I:	8	3
Stage II:	24	10

With this design, we have no more than a 5% chance of concluding effective (≥60% success rate) when the success rate is at most 30%. Similarly, we have no more than a 20% chance of concluding ineffective (≤30% stabilization rate) when it is effective. If the actual response rate is 30% or worse, we have at least a 0.81 probability that the trial will stop after the first 8 subjects. If the response rate is ≥60%, the probability that the trial will be stopped in stage I is 0.17. The overall power of this design is 80.6%.

The first stage of study requires 8 patients in each cohort. If 3 or fewer patients achieve stabilization or regression of hepatic metastases, further enrollment of patients into that group will be stopped with the conclusion that clinical benefit rate can not be 60% or greater. If at least 4 patients show response (CR, PR, or SD) in at least one of these two groups, the study will be continued to accrue 16 more patients in that group(s). If 10 or fewer patients among 24 patients in the group(s) show stabilization or regression of hepatic metastases, the study will be concluded that clinical benefit rate will not be 60% or more and no further study will be considered for the patients from the corresponding group(s).

We are currently treating 50-100 new uveal melanoma patients with hepatic metastases at Thomas Jefferson University Hospital. Approximately 1-2 patients would be accrued to this study every months and the accrual to this study will be completed within 2 years.

The estimated median survival of patients who receive this treatment is 6-12 months. We will follow the patients until their death or for 2 years. A maximum of 4 years including accrual of patients will be required to complete this study.

The overall survival (OS), progression free survival without progression of hepatic metastasis (PFS-L), and duration of response will be evaluated using the Kaplan-Meier method. All estimates of rates (e.g., clinical benefit rate and toxicity) will be presented with corresponding confidence intervals. For clinical benefit rates, the method of Atkinson and Brown will be used to adjust for the two-stage design (44).

All molecular and immunological studies are exploratory and the results will be reported in a descriptive manner. The genome-wide SNP analysis will be done at the KCC core facility. Paired

t-test or paired Wilcoxon test will be used to compare the serum cytokines and T cell subpopulations before and after radiosphere treatment.

13.0 Data Disclosure and Patient Confidentiality

Patient medical information obtained as a result of this study is considered confidential. Disclosure to third parties other than to the patient's primary physician and to those noted below is prohibited. All reports and communications relating to subjects in this study will identify each patient only by his/her number (and initials if allowed by local regulations). Data generated as a result of this study must be available for inspection upon request by the FDA (or other Health Authority or government agency), and the IRB. In addition, Sirtex Medical Inc. that provides the funding for this clinical study has a right to investigate the data obtained from this study.
Patient confidentiality will be maintained at all times unless government regulation or applicable law requires disclosure. If local or national regulations are modified to require additional consent(s) or documentation for release of source documents or other medical information, required for the study, it is the responsibility of the investigator to obtain such consent and documentation with relevant approvals by the local IRB.

14.0 Ethical Considerations

14.1 Protection of Human Subjects from Research Risks

The study will be conducted in accordance with the Declaration of Helsinki and with rules and regulations in accord with the U.S. Office of Protection from Research Risks (OPRR).

14.2 Institutional Review Board

The study will be reviewed and approved by a duly constituted IRB before patients are screened for entry. The investigator will ensure that all aspects of the IRB review are conducted in accordance with current institutional, local, and national regulations. Amendments to the protocol will be subject to the same requirements as the original protocol. The Investigator will submit all periodic reports and updates that the IRB may require, including any final close out reports. The Investigator will inform the IRB of any reportable adverse events as required by local regulations.

14.3 Informed Consent

Each patient will be provided with oral and written information that describes the nature and duration of the study in a language he/she can understand. The required schedule for treatment and interval safety evaluations (irrespective of holidays), plus the planned follow-up schedule, should be carefully reviewed and possibly reinforced with a calendar. The patient must consent in writing to participate before undergoing therapy on the protocol. The original signed consent form will be retained. Each patient will be given a copy of his/her consent form.

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